

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
4 December 2003 (04.12.2003)

PCT

(10) International Publication Number
WO 03/099824 A1

(51) International Patent Classification⁷: C07D 491/04,
A61K 31/407, A61P 31/12 // C07M 7:00

(74) Agents: DIANA, Leonard, P. et al.; Fitzpatrick, Cella,
Harper & Scinto, 30 Rockefeller Plaza, New York, NY
10112-3801 (US).

(21) International Application Number: PCT/US03/15823

(22) International Filing Date: 21 May 2003 (21.05.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/382,148 21 May 2002 (21.05.2002) US

(71) Applicants: WYETH [US/US]; Five Giralda Farms,
Madison, NJ 07940 (US). VIROPHARMA INCORPO-
RATED [US/US]; 405 Eagleview Boulevard, Exton, PA
19341 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU,
ZA, ZM, ZW.

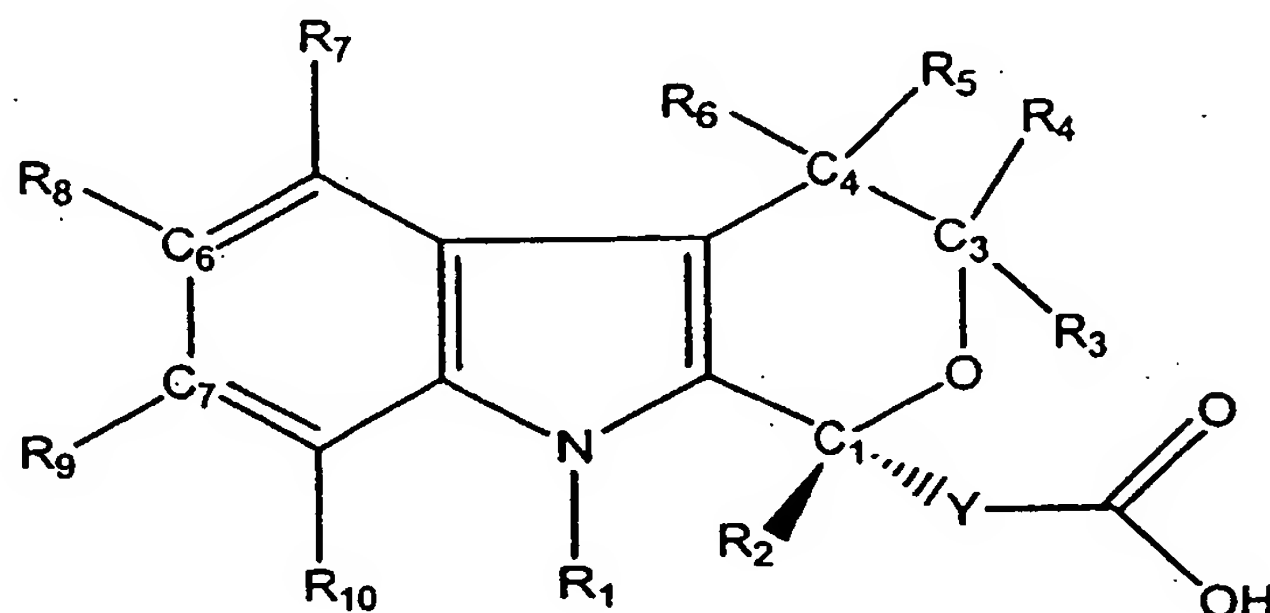
(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: R-ENANTIOMERS OF PYRANOINDOLE DERIVATIVES AGAINST HEPATITIS C



(57) Abstract: The invention is directed to
a compound and a pharmaceutical composi-
tion of the formula: Wherein substitutions at
R₁, R₂, R₃ - R₁₂, and Y are set forth in the
specification.

WO 03/099824 A1

R-ENANTIOMERS OF PYRANOINDOLE DERIVATIVES AGAINST HEPATITIS C

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention is directed to pharmaceutical compositions containing stereoisomers of pyranoindole derivatives and processes for their preparation.

10 Related Background art

[0002] Hepatitis C is a common viral infection that can lead to chronic Hepatitis, cirrhosis, liver failure, and hepatocellular carcinoma. Infection with the Hepatitis C virus (HCV) leads to chronic Hepatitis in at least 85% of cases, is the leading reason for liver transplantation, and is responsible for at least 10,000 deaths annually in the United States (Hepatology, 1997, 26 (Suppl. 1), 2S-10S).

[0003] The Hepatitis C virus is a member of the *Flaviviridae* family, and the genome of HCV is a single-stranded linear RNA of positive sense (Hepatology, 1997, 26 (Suppl. 1), 11S-14S). HCV displays extensive genetic heterogeneity; at least 6 genotypes and more than 50 subtypes have been identified.

20 [0004] There is no effective vaccine to prevent HCV infection. The only therapy currently available is treatment with interferon- α (INF- α) or combination therapy of INF- α with the nucleoside analog ribavirin (Antiviral Chemistry and Chemotherapy, 1997, 8, 281-301). However, only about 40% of treated patients

develop a sustained response, so there is a need for more effective anti-HCV therapeutic agents.

[0005] The HCV genome contains a number of non-structural proteins: NS2, NS3, NS4A, NS4B, NS5A, and NS5B (J. General Virology, 2000, 81, 1631-1648).

- 5 NS5B is a RNA-dependent RNA polymerase which is essential for viral replication, and therefore, the inhibition of NS5B is a suitable target for the development of therapeutic agents.

[0006] In the following US patents, pyranoindole derivatives are disclosed and the compounds are stated to have antidepressant and antiulcer activity: 3,880,853
10 (4/29/75), 4,118,394 (10/3/78). In US patent 4,179,503 (12/18/79) pyranoindoles are disclosed and stated to have diuretic activity. In the following US patents, pyranoindole derivatives are disclosed and the compounds are stated to have antiinflammatory, analgesic, antibacterial, and antifungal activity: 3,843,681
(10/22/74), 3,939,178 (2/17/76), 3,974,179 (8/10/76), 4,070,371 (1/24/78),
15 4,076,831 (2/28/78). In the following US patents, pyranoindole derivatives are disclosed and the compounds are stated to have antiinflammatory and analgesic activity: 4,670,462 (6/2/87), 4,686,213 (8/11/87), 4,785,015 (11/15/88), 4,810,699
(3/7/89), 4,822,781 (4/18/89), 4,960,902 (10/2/90). In US patent 5,776,967
(7/7/98), and US patent 5,830,911 (11/3/98), pyranoindole derivatives are disclosed
20 and the compounds are said to inhibit cyclooxygenase-2 and be useful for treating arthritic disorders, colorectal cancer, and Alzheimer's disease.

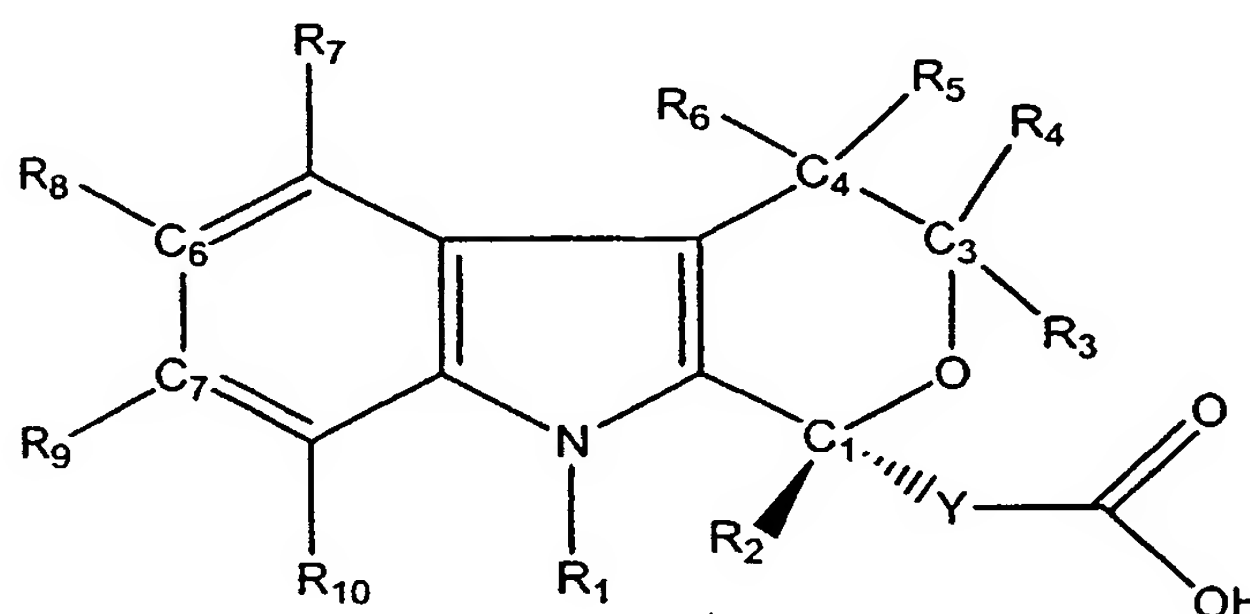
[0007] Also, in the following US patents, processes for preparing pyranoindole derivatives are disclosed: 4,012,417 (3/15/77), 4,036,842 (7/19/77), 4,585,877
(4/29/86), 4,822,893 (4/18/89). Processes for the resolution of racemic
25 pyranoindole derivatives are disclosed in the following US patents: 4,501,899
(2/26/85), 4,515,961 (5/7/85), 4,520,203 (5/28/85), 4,544,757 (10/1/85).

[0008] US provisional patent application No. 60/382,148, filed May 21, 2002, and which is hereby incorporated by reference in its entirety, provides other examples of compounds.

BRIEF SUMMARY OF THE INVENTION

[0009] This invention relates to a pharmaceutical composition comprising stereoisomers of pyranoindole derivatives, processes for their preparation, and pharmaceutical compositions containing them and to their use in the treatment of
 5 Hepatitis C viral infection.

[0010] In accordance with this invention there is provided a pharmaceutical composition comprising a compound represented by formula (A):



wherein:

[0011] R₁ is H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3
 10 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or an arylalkyl or an alkylaryl of 7 to 12 carbon atoms;

[0012] R₂ is H, a straight chain alkyl of 1 to 12 carbon atoms, a branched alkyl of
 15 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, an alkoxyalkyl of 2 to 12 carbon atoms, an arylalkyl or alkylaryl of 7 to 12 carbon atoms, a cyanoalkyl of 1 to 8 carbon atoms, an alkylthioalkyl of 2 to 16 carbon atoms, a cycloalkyl-alkyl of 4 to 24 carbon atoms, a substituted or unsubstituted aryl, or a heteroaryl;

[0013] R₃ – R₆ are independently H, a straight chain alkyl of 1 to 8 carbon atoms,
 20 a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, a substituted or unsubstituted aryl, furanylmethyl, arylalkyl or alkylaryl of 7 to 12 carbon atoms, alkynyl of 2 to 7 carbon atoms, or R₅ and R₆ together with the ring carbon atom to which they are attached form a carbonyl group;

- [0014] $R_7 - R_{10}$ are independently H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbons atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, furanylmethyl, arylalkyl or alkylaryl of 7 to 12 carbon atoms, alkynyl of 2 to 7 carbon atoms, phenylalkynyl, alkoxy of 1 to 8 carbon atoms, arylalkoxy of 7 to 12 carbon atoms, alkylthio of 1 to 8 carbon atoms, trifluoromethoxy, trifluoroethoxy, trifluoromethylthio, trifluoroethylthio, acyl of 1 to 7 carbon atoms, COOH, COO-alkyl, $\text{CONR}_{11}\text{R}_{12}$, F, Cl, Br, I, CN, CF_3 , NO_2 , alkylsulfinyl of 1 to 8 carbon atoms, alkylsulfonyl of 1 to 6 carbon atoms, pyrrolidinyl, or thiazolidinyl;
- [0015] $R_{11} - R_{12}$ are independently H, straight chain alkyl of 1 to 8 carbon atoms, branched alkyl of 3 to 12 carbon atoms, cycloalkyl of 3 to 12 carbon atoms, a substituted or unsubstituted aryl or heteroaryl;
- [0016] Y is a bond, CH_2 , CH_2CH_2 , aryl, or R_2 and Y together with the ring carbon atom to which they are attached may additionally form a spirocyclic cycloalkyl ring of 3 to 8 carbon atoms; or
- [0017] a crystalline form or a pharmaceutically acceptable salt thereof; and
- [0018] a pharmaceutically acceptable carrier.
- [0019] In one embodiment of the invention the pharmaceutical compositions comprise: (R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid; (R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid; (R)-5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid; or (R)-5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid.
- [0020] For purposes of this invention the term "alkyl" includes both straight and branched alkyl moieties, preferably of 1 to 8 carbon atoms. The term "alkenyl" refers to a radical aliphatic hydrocarbon containing one double bond and includes both straight and branched alkenyl moieties of 2 to 7 carbon atoms. Such alkenyl moieties may exist in the E or Z configurations; the compounds of this invention include both configurations. The term "alkynyl" includes both straight chain and branched moieties containing 2 to 7 carbon atoms having at least one triple bond. The term "cycloalkyl" refers to alicyclic hydrocarbon groups having 3 to 12 carbon atoms and includes but is not limited to: cyclopropyl, cyclobutyl, cyclopentyl,

- cyclohexyl, cycloheptyl, norbornyl, or adamantyl. For purposes of this invention the term "aryl" is defined as an aromatic hydrocarbon moiety and may be substituted or unsubstituted. An aryl may be selected from but not limited to, the group: phenyl, α -naphthyl, β -naphthyl, biphenyl, anthryl, tetrahydronaphthyl, phenanthryl, fluorenyl, indanyl, biphenylenyl, acenaphthenyl, acenaphthylenyl, or phenanthrenyl groups. In one embodiment the substituted aryl may be optionally mono-, di-, tri- or tetra-substituted with substituents selected from, but not limited to, the group consisting of alkyl, acyl, alkoxycarbonyl, alkoxy, alkoxyalkyl, alkoxyalkoxy, cyano, halogen, hydroxy, nitro, trifluoromethyl, trifluoromethoxy, trifluoropropyl, amino, alkylamino, dialkylamino, dialkylaminoalkyl, hydroxyalkyl, alkoxyalkyl, alkylthio, $-\text{SO}_3\text{H}$, $-\text{SO}_2\text{NH}_2$, $-\text{SO}_2\text{NHalkyl}$, $-\text{SO}_2\text{N(alkyl)}_2$, $-\text{CO}_2\text{H}$, CO_2NH_2 , $\text{CO}_2\text{NHalkyl}$, and $-\text{CO}_2\text{N(alkyl)}_2$. Preferred substituents for aryl and heteroaryl include: alkyl, halogen, amino, alkylamino, dialkylamino, trifluoromethyl, trifluoromethoxy, arylalkyl, and alkylaryl.
- 15 [0021] For purposes of this invention the term "heteroaryl" is defined as an aromatic heterocyclic ring system (monocyclic or bicyclic) where the heteroaryl moieties are five or six membered rings containing 1 to 4 heteroatoms selected from the group consisting of S, N, and O, and include but is not limited to: (1) furan, thiophene, indole, azaindole, oxazole, thiazole, isoxazole, isothiazole, imidazole, N-methylimidazole, pyridine, pyrimidine, pyrazine, pyrrole, N-methylpyrrole, pyrazole, N-methylpyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1-methyl-1,2,4-triazole, 1H-tetrazole, 1-methyltetrazole, benzoxazole, benzothiazole, benzofuran, benzisoxazole, benzimidazole, N-methylbenzimidazole, azabenzimidazole, indazole, quinazoline, quinoline, pyrrolidinyl; (2) a bicyclic aromatic heterocycle where a phenyl, pyridine, pyrimidine or pyridazine ring is: (i) fused to a 6-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom; (ii) fused to a 5 or 6-membered aromatic (unsaturated) heterocyclic ring having two nitrogen atoms; (iii) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one nitrogen atom together with either one oxygen or one sulfur atom; or (iv) fused to a 5-membered aromatic (unsaturated) heterocyclic ring having one heteroatom selected from O, N or S.
- 20
- 25
- 30

[0022] For the purposes of this invention the term "alkoxy" is defined as C1-C12alkyl-O-; the term "aryloxy" is defined as aryl-O-; the term "heteroaryloxy" is defined as heteroaryl-O-; wherein alkyl, aryl, and heteroaryl are as defined above.

[0023] For purposes of this invention the term "arylalkyl" is defined as aryl-C1-C6-alkyl-; arylalkyl moieties include benzyl, 1-phenylethyl, 2-phenylethyl, 3-phenylpropyl, 2-phenylpropyl and the like.

[0024] For purposes of this invention the term "alkylaryl" is defined as C1-C6-alkyl-aryl-.

[0025] For purposes of this invention the term "alkylthio" is defined as C1-C6-alkyl-S-.

[0026] For purposes of this invention "alkoxyalkyl," "cycloalkyl-alkyl," "alkylthioalkyl," "aryloxyalkyl," and "heteroaryloxyalkyl" denote an alkyl group as defined above that is further substituted with an alkoxy, cycloalkyl, alkylthio, aryloxy, or heteroaryloxy group as defined above.

[0027] For purposes of this invention "arylalkoxy," "alkoxyalkoxy," "alkylthioalkoxy," and "heteroarylalkoxy" denote an alkoxy group as defined above that is further substituted with an aryl, alkoxy, alkylthio, or heteroaryl group as defined above.

[0028] For purposes of this invention "arylthio" and "heteroarylthio," denote a thio group that is further substituted with an aryl or heteroaryl group as defined above.

[0029] For purposes of this invention "arylthioalkyl" and "heteroarylthioalkyl" denote an alkyl group as defined above that is further substituted with an arylthio or heteroarylthio group as defined above.

[0030] For purposes of this invention the term "arylalkylthio" is defined as aryl-C1-C8-alkyl-S-; "heteroarylalkylthio" is defined as heteroaryl-C1-C8-alkyl-S-, where aryl and heteroaryl are as defined above.

[0031] For purposes of this invention "aryloxyalkylthio" is defined as aryloxy-C1-C8-alkyl-S; "heteroaryloxyalkylthio" is defined as heteroaryloxy-C1-C8-alkyl-S-; where aryloxy, heteroaryloxy, and alkyl are defined above.

[0032] For purposes of this invention "phenylalkynyl" is an alkynyl group further substituted with a phenyl group.

[0033] In the most preferred embodiment of this invention a substituted methyl comprises a methyl substituent further substituted with for example a furanyl

group. In another embodiment of this invention a furanyl substituent is further substituted with a methyl group.

[0034] In a preferred embodiment of this invention trifluoromethoxy includes but is not limited to $\text{CF}_3\text{O}-$. In another embodiment of this invention

5 trifluoromethylthio includes but is not limited to $\text{CF}_3\text{S}-$.

[0035] In one embodiment of this invention trifluoroethoxy is $\text{CF}_3\text{CH}_2\text{O}-$. In another embodiment of this invention trifluoroethylthio is $\text{CF}_3\text{CH}_2\text{S}-$.

[0036] The terms "monoalkylamino" and "dialkylamino" refer to moieties with one or two alkyl groups wherein the alkyl chain is 1 to 8 carbons and the groups

10 may be the same or different. The terms monoalkylaminoalkyl and dialkylaminoalkyl refer to monoalkylamino and dialkylamino moieties with one or two alkyl groups (the same or different) bonded to the nitrogen atom which is attached to an alkyl group of 1 to 8 carbon atoms.

[0037] "Acyl" is a radical of the formula $-(\text{C}=\text{O})\text{-alkyl}$ or $-(\text{C}=\text{O})\text{-perfluoroalkyl}$ wherein the alkyl radical or perfluoroalkyl radical is 1 to 7 carbon atoms; preferred examples include but are not limited to, acetyl, propionyl, butyryl, trifluoroacetyl.

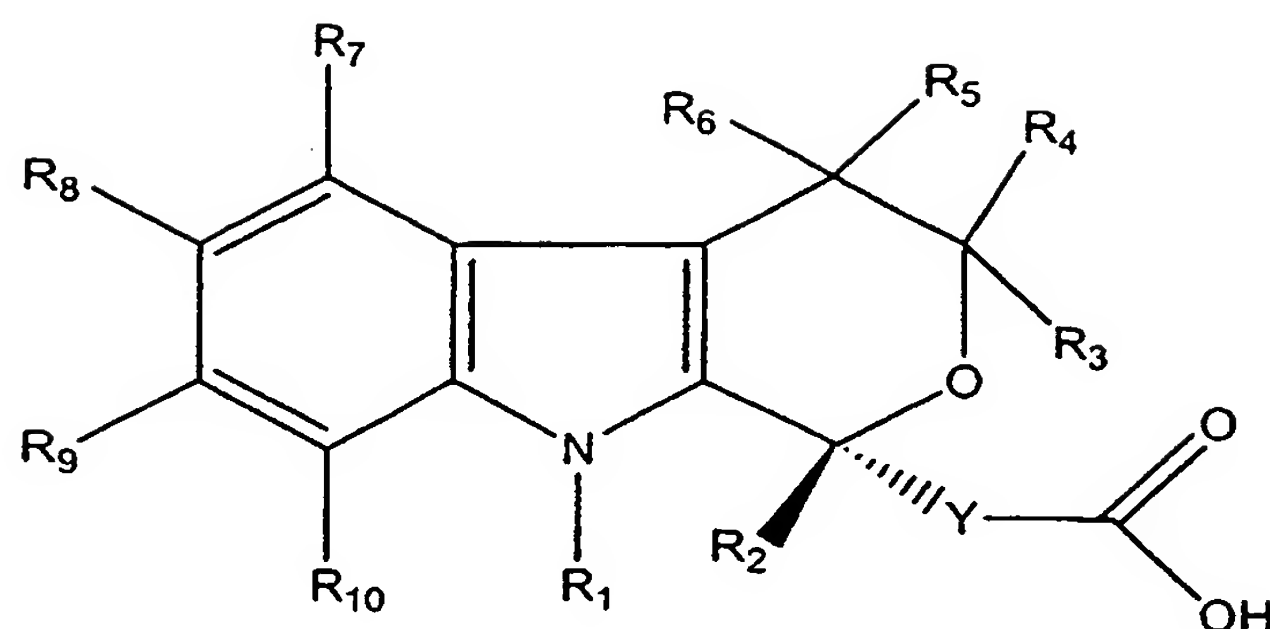
[0038] For purposes of this invention alkylsulfinyl is a $\text{R}'\text{SO}-$ radical, where R' is an alkyl radical of 1-8 carbon atoms. Alkylsulfonyl is a $\text{R}'\text{SO}_2-$ radical, where R' is an alkyl radical of 1-8 carbon atoms. Alkylsulfonamido, alkenylsulfonamido, 20 alkynylsulfonamido are $\text{R}'\text{SO}_2\text{NH}-$ radicals, where R' is an alkyl radical of 1-8 carbon atoms, an alkenyl radical of 2-8 carbon atoms, or an alkynyl radical of 2-8 carbon atoms, respectively.

[0039] Saturated or partially saturated heteroaryl groups are defined in this invention as heterocyclic rings selected from but not limited to the moieties; 25 azetidiny, 1,4-dioxanyl, hexahydroazepiny, piperaziny, piperidiny, pyrrolidiny, morpholiny, thiomorpholiny, dihydrobenzimidazolyl, dihydrobenzofuranyl, dihydrobenzothienyl, dihydrobenzoxazolyl, dihydrofuranyl, dihydroimidazolyl, dihydroindolyl, dihydroisooxazolyl, dihydroisothiazolyl, dihydrooxadiazolyl, dihydrooxazolyl, dihydropyrraziny, dihydropyrazolyl, dihydropyridiny, 30 dihydropyrimidiny, dihydropyrroly, dihydroquinoliny, dihydrotetrazolyl, dihydrothiadiazolyl, dihydrothiazolyl, dihydrothienyl, dihydrotriazolyl, dihydroazetidiny, dihydro-1,4-dioxanyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydroquinoliny, and tetrahydroisoquinoliny.

[0040] The compounds of this invention contain one or more asymmetric carbon atoms and may thus give rise to stereoisomers, such as enantiomers and diastereomers. The stereoisomers of the instant invention are named according to the Cahn-Ingold-Prelog System. While the C₁ carbon of formula (A) is specified
5 the present invention includes all the other possible stereoisomers; as well as the racemic mixtures and other mixtures of R and S stereoisomers (scalemic mixtures which are mixtures of unequal amounts of enantiomers) and pharmaceutically acceptable salts thereof. It should be noted that stereoisomers of the invention having the same relative configuration at a chiral center may nevertheless have
10 different R and S designations depending on the substitution at the indicated chiral center.

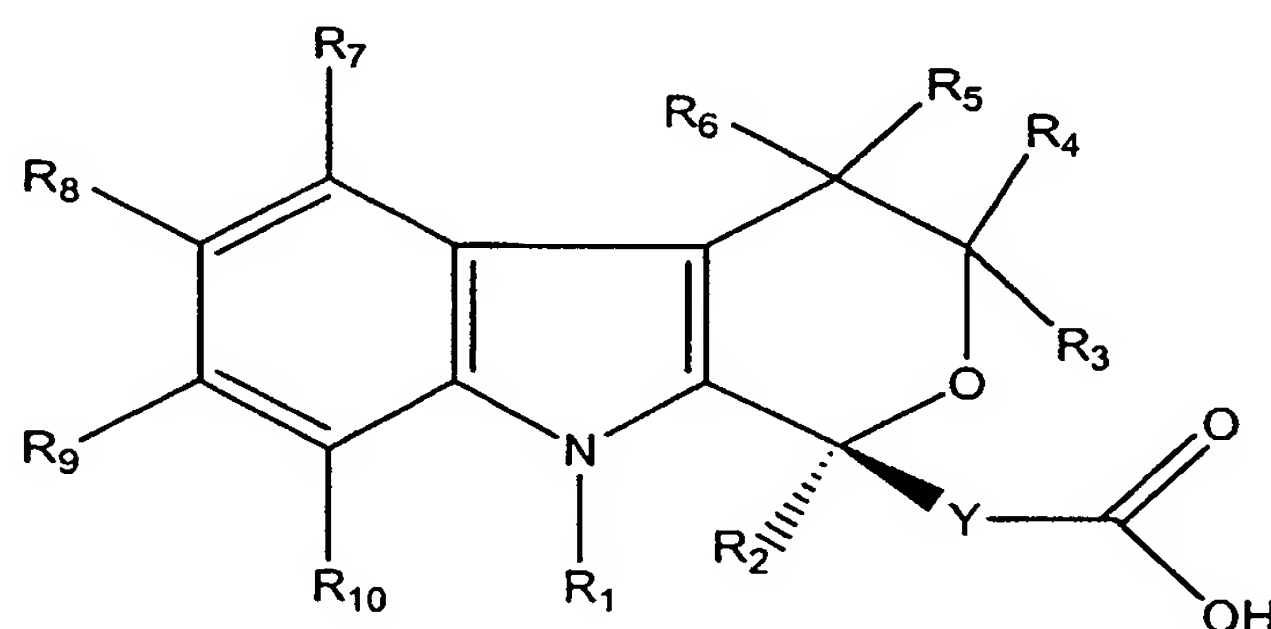
[0041] For compounds of this invention containing two chiral centers, four possible stereoisomers are possible; these four stereoisomers are classified as two racemic pairs of diastereomers. These compounds of the invention may be present
15 as racemic diastereomers which would be designated following the convention described in the 1997 Chemical Abstracts Index Guide, Appendix IV (Columbus, OH) whereas the first cited chiral atom is designated R* and the next cited chiral atom is designated R* if it possesses the same chirality as the first cited stereocenter or S* if it possesses opposite chirality to the first cited stereocenter.
20 Alternatively, these compounds of the invention may be present as non-racemic mixtures of two diastereomers owing to the existence of a predefined stereocenter. In these instances, the predefined stereocenter is assigned based on the Cahn-Ingold-Prelog System and the undefined stereocenter is designated R* to denote a mixture of both R and S stereoisomers at this center. Compounds of this invention
25 which possess two chiral centers but which are present as single stereoisomers are described using the Cahn-Ingold-Prelog System.

[0042] Based on the chiral center at the C₁ carbon position, a preferred embodiment of the instant invention is the compound of formula A(a) shown below:



A(a)

[0043] The configuration at C₁ in Formula A(a) for purposes of this invention is also referred to as "Isomer A", and the opposite configuration at C₁ is herein defined as "Isomer B" and has the formula A(b) shown below:



A(b)

5

[0044] In one embodiment of this invention the compound of the invention is comprised of a ratio of Isomer A to Isomer B of greater than 1:1. In the most preferred embodiment the compound is comprised of 100% Isomer A. In further embodiments the compound is comprised of a ratio of Isomer A to Isomer B of at least about 9:1. In another embodiment the compound is comprised of a ratio of Isomer A to Isomer B of at least about 8:1. Additionally the compound is comprised of a ratio of Isomer A to Isomer B of at least about 7:1.

[0045] Pharmaceutically acceptable salts of the compounds of formula (I) having acidic moieties at R₃, R₄, R₅, R₆, R₇, R₈, R₉, or R₁₀ may be formed from organic and inorganic bases. For example alkali metal salts: sodium, lithium, or potassium and N- tetraalkylammonium salts such as N-tetrabutylammonium salts. Similarly, when a compound of this invention contains a basic moiety at R₃, R₄, R₅, R₆, R₇,

15

R₈, R₉, or R₁₀, salts can be formed from organic and inorganic acids. For example salts can be formed from acetic, propionic, lactic, citric, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, phthalic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, methanesulfonic, naphthalenesulfonic, benzenesulfonic,

5 toluenesulfonic, camphorsulfonic, and similarly known acceptable acids.

[0046] The compounds are preferably provided orally or subcutaneously. The compounds may be provided by intralesional, intraperitoneal, intramuscular or intravenous injection; infusion; liposome-mediated delivery; topical, nasal, anal, vaginal, sublingual, urethral, transdermal, intrathecal, ocular or otic delivery. In
10 order to obtain consistency in providing the compound of this invention it is preferred that a compound of the invention is in the form of a unit dose. Suitable unit dose forms include tablets, capsules and powders in sachets or vials. Such unit dose forms may contain from 0.1 to 100 mg of a compound of the invention and preferably from 2 to 50 mg. Still further preferred unit dosage forms contain 5 to
15 25 mg of a compound of the present invention. The compounds of the present invention can be administered orally at a dose range of about 0.01 to 100 mg/kg or preferably at a dose range of 0.1 to 10 mg/kg. Such compounds may be administered from 1 to 6 times a day, more usually from 1 to 4 times a day. The effective amount will be known to one of skill in the art; it will also be dependent
20 upon the form of the compound. One of skill in the art could routinely perform empirical activity tests to determine the bioactivity of the compound in bioassays and thus determine what dosage to administer.

[0047] The compounds of the invention may be formulated with conventional excipients, such as a filler, a disintegrating agent, a binder, a lubricant, a flavoring
25 agent, a color additive, or a carrier. The carrier may be for example a diluent, an aerosol, a topical carrier, an aqueous solution, a nonaqueous solution or a solid carrier. The carrier may be a polymer or a toothpaste. A carrier in this invention encompasses any of the standard pharmaceutically accepted carriers, such as phosphate buffered saline solution, acetate buffered saline solution, water,
30 emulsions such as an oil/water emulsion or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules.

[0048] When provided orally or topically, such compounds would be provided to a subject by delivery in different carriers. Typically, such carriers contain excipients

such as starch, milk, sugar, certain types of clay, gelatin, stearic acid, talc, vegetable fats or oils, gums, or glycols. The specific carrier would need to be selected based upon the desired method of delivery, for example, phosphate buffered saline (PBS) could be used for intravenous or systemic delivery and vegetable fats, creams, salves, ointments or gels may be used for topical delivery.

[0049] The compounds of the present invention may be delivered together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in treatment or prevention of Hepatitis C viral infection. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (for example, Tris-HCl, acetate, phosphate), pH and ionic strength, additives such as albumins or gelatin to prevent absorption to surfaces, detergents (for example, TWEEN 20, TWEEN 80, PLURONIC F68, bile acid salts), solubilizing agents (for example, glycerol, polyethylene glycerol), anti-oxidants (for example ascorbic acid, sodium metabisulfate), preservatives (for example, thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (for example, lactose, mannitol), covalent attachment of polymers such as polyethylene glycol, complexation with metal ions, or incorporation of the compound into or onto particulate preparations of hydrogels or liposomes, micro-emulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance of the compound or composition. The choice of compositions will depend on the physical and chemical properties of the compound capable of treating or preventing a Hepatitis C viral infection.

[0050] The compound of the present invention may be delivered locally via a capsule that allows a sustained release of the compound over a period of time. Controlled or sustained release compositions include formulation in lipophilic depots (for example, fatty acids, waxes, oils).

[0051] For purposes of this invention a chiral amine comprises a nitrogen atom in a three-membered ring connected to another atom bearing an unshared pair of electrons and may be, but is not limited to, ephedrine hemihydrate or cinchomine.

[0052] Another embodiment of this invention is where R2 of formula (A) is a sec-butyl group. In a preferred embodiment, the chiral carbon of the sec-butyl group

has an S to R configuration ratio of 1:1. In further embodiments, the chiral carbon of the sec-butyl group has an S to R configuration ratio selected from the group consisting of at least 7:1, at least 8:1, and at least 9:1. In a most preferred embodiment of the invention, the chiral carbon of the sec-butyl group has 100% S configuration.

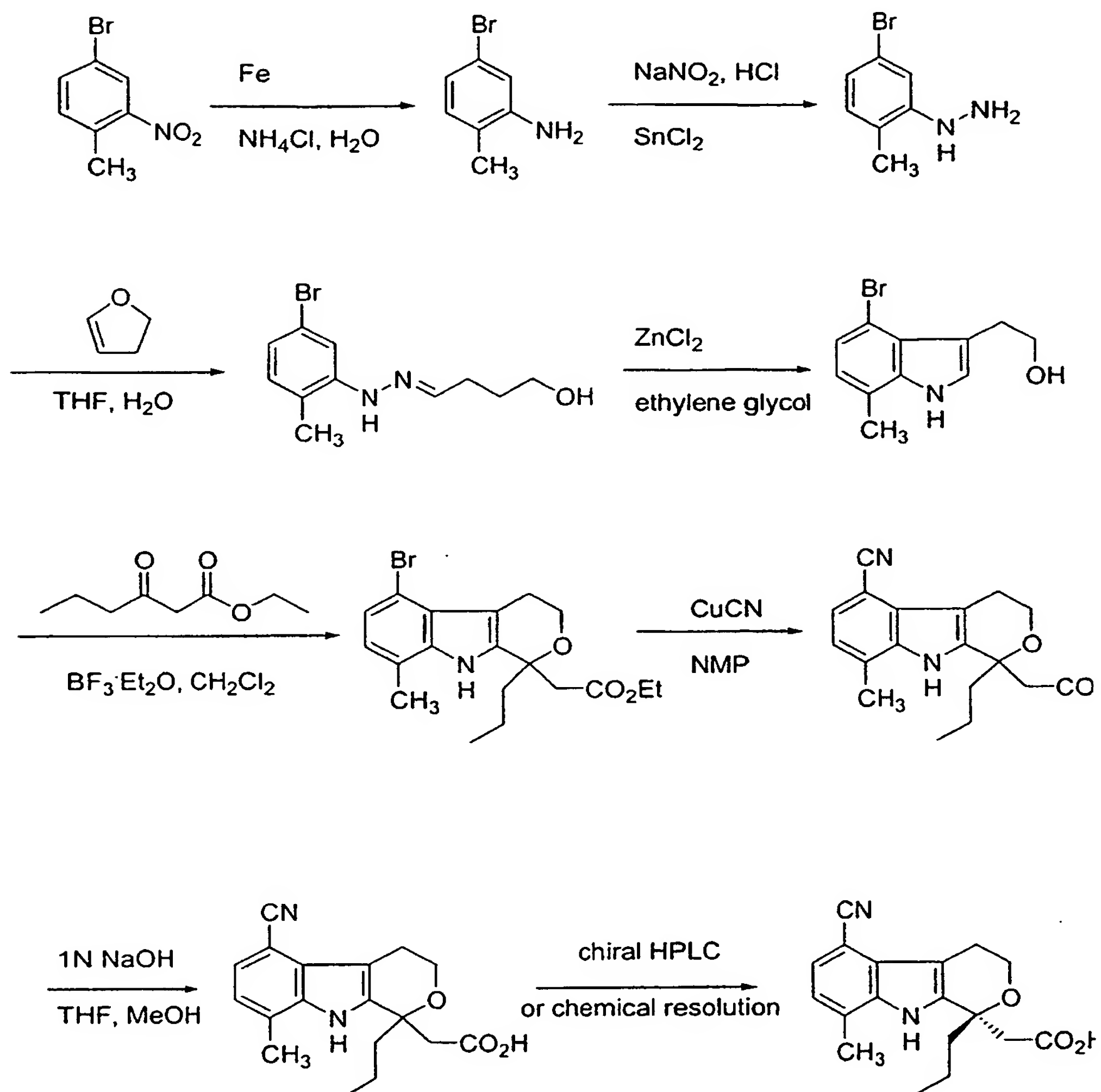
[0053] The following experimental details are set forth to aid in an understanding of the invention, and are not intended, and should not be construed, to limit in any way the invention set forth in the claims that follow thereafter.

DETAILED DESCRIPTION OF THE INVENTION

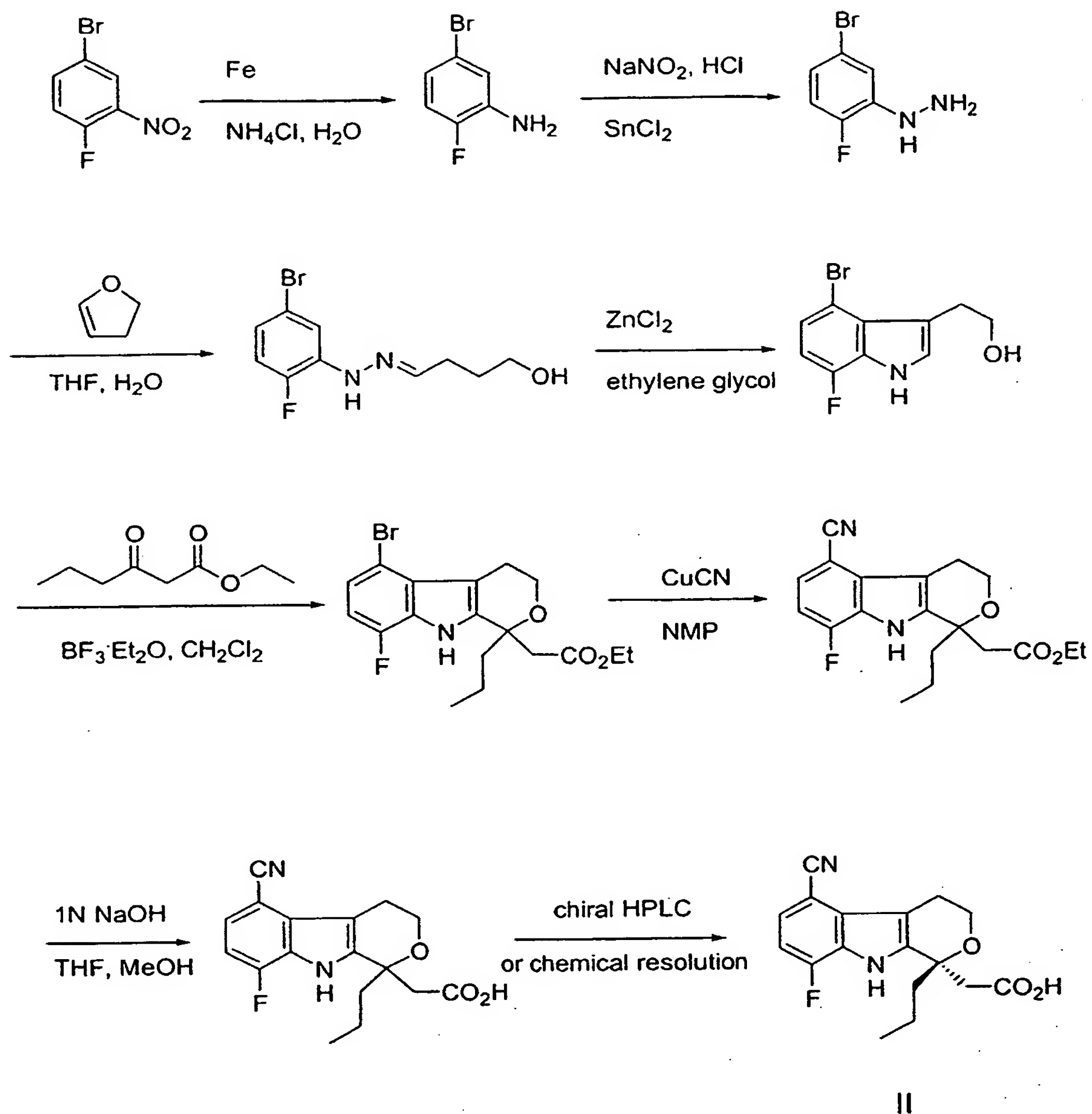
[0054] The compounds and compositions of the present invention can be readily prepared according to the following reaction schemes or modification thereof. It is also possible to make use of variants of these steps, which in themselves are known to and well within the preparatory skill of the medicinal chemist. Optically active isomers may be prepared, for example, by resolving racemic derivatives or by asymmetric synthesis. The resolution can be carried out by methods known to those skilled in the art such as in the presence of a resolving agent, by chromatography, or combinations thereof.

[0055] The compounds of the present invention can be synthesized as described in the schemes below (Schemes 1-4).

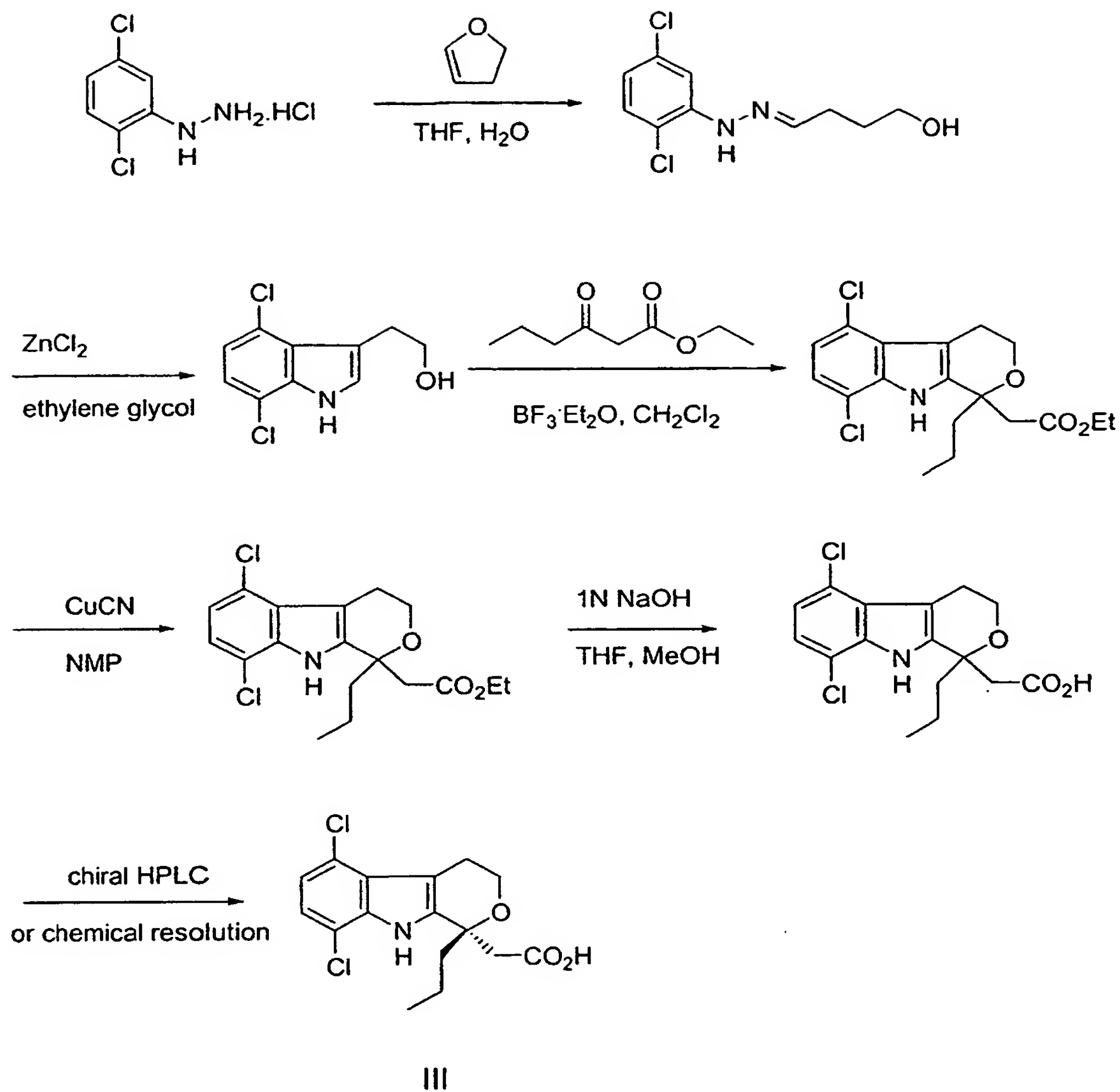
Scheme 1



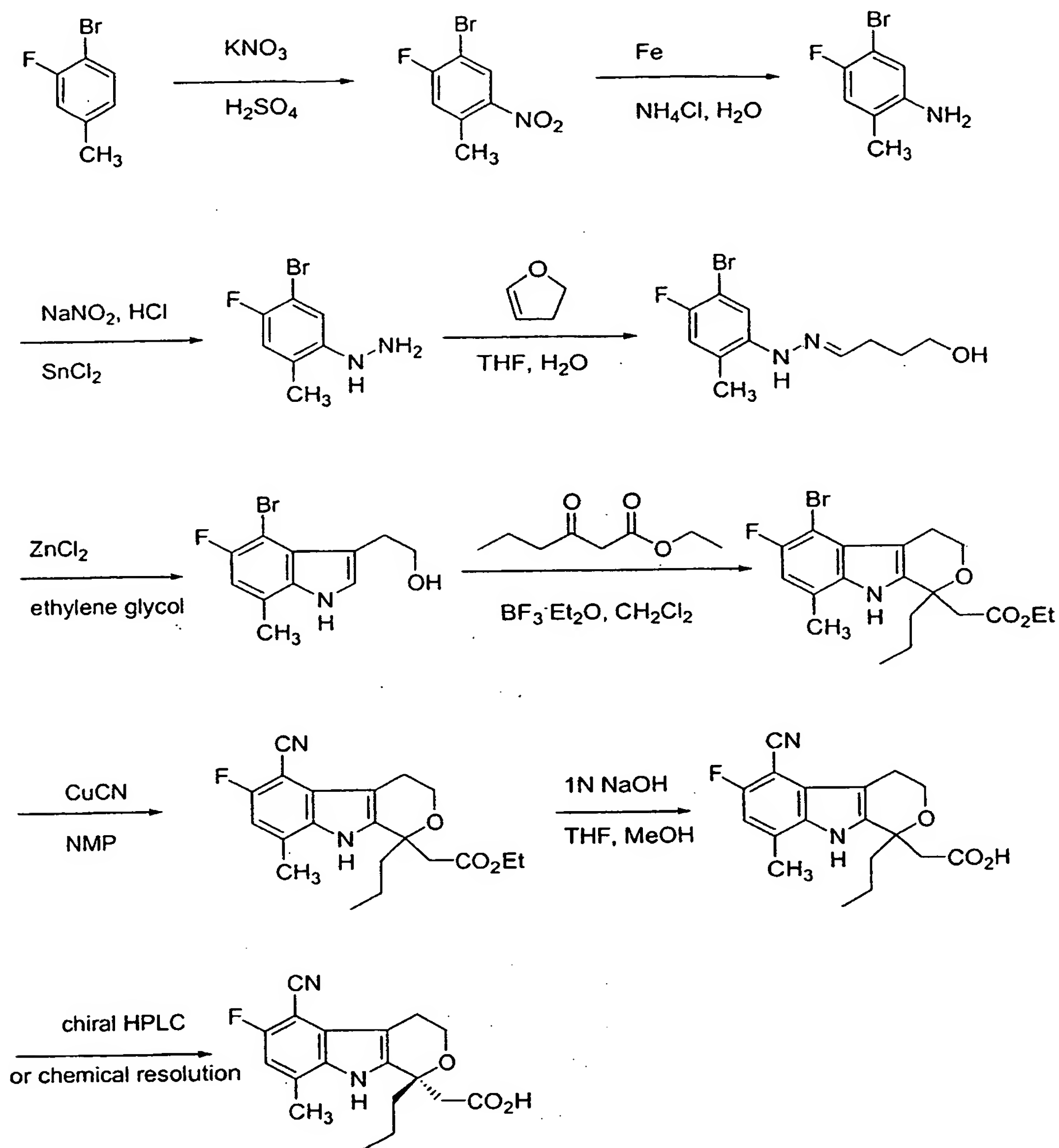
Scheme 2



Scheme 3



Scheme 4



IV

[0056] The ability of the compounds of the present invention to inhibit Hepatitis C

5 Polymerase was established by the following experimental procedure:

[0057] NS5B from the BK strain (1b subtype) is expressed in *E. coli* as a protein in which the 21 C-terminal amino acids are replaced with a short linker and a hexahistidine tag (GSHHHHHH). The purified protein is mixed with radioactive

nucleotides and allowed to replicate a heteropolymeric RNA substrate, primed by an endogenous short hairpin, resulting in an approximately 760 nt product. The radioactive product is captured on a filter and quantitated after removal of the unincorporated nucleotides.

5 Reagents:

10 mM uridine 5'-triphosphate (UTP) (Promega # p116B)

10 mM adenine 5'-triphosphate (ATP) (Promega # p113B)

10 mM cytidine 5'-triphosphate (CTP) (Promega # p114B)

10 mM guanine 5'-triphosphate (GTP) (Promega # p115B)

10 bovine Serum Albumin (BSA) 10 mg/ml NEB (100X at 10 mg/ml) #007-BSA
RNasein (Promega #N251X) 40 U/ul

³³P-GTP (NEN-easytides NEG/606H 3000 Ci/mmol, 370 MBq/ml, 10 mCi/ml)

Falcon polypropylene 96 well plates (Becton Dickinson # 351190)

Millipore Multiscreen assay system-96 well-filtration plate #MADE NOB 50

15 Optiphase Supermix (Wallac) formulated by Fisher

Millipore Multiscreen liner for use in microbeta 1450-106 cassette (Wallac) Perkin
Elmer #1450-433

1 M (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) HEPES, pH 7.3

Amersham Pharmacia Biotec (US16924-500 ml)

20 1 M MgCl₂ (SIGMA #M1028)

Dithiothreitol (DTT) (solid) (SIGMA # D9779)

RNase free water (GIBCO-BRL #10977-023)

Dimethyl sulfoxide (Aldrich #27685-5)

Basilen Blue (Sigma, B5520)

25 0.5M ethylenediaminetetraacetic acid (EDTA), pH 8 (GIBCO-BRL #15575-020)

Dibasic sodium phosphate 7 hydrate (Na₂HPO₄·7H₂O; Baker#3824-07)

Phosphoric acid (Baker, #0262.02)

[0058] Further reagent preparation:

0.5 M Na Phosphate buffer. Per liter, weigh 134 gr Na₂HPO₄·7H₂O, add water to

30 900 ml. Adjust pH to 7.0 with phosphoric acid. Top off with water to 1 L.

Dilute nucleotides 1:1000 to 10 μM (GTP and CTP) or 1:100 to 100 μM (ATP and
UTP) into RNA-se free water.

[0059] Procedure:

(1) Compounds 10 µl at 10 µg/ml in 15 % dimethylsulfoxide (DMSO)

When starting from 100 µg/ml compound stock in 1% DMSO:

Dispense 5 µl 30 % DMSO per well

- 5 Dispense 5 µl compound (100 µg/ml) per well.

When starting from 50 µg/ml compound stock in 15 % DMSO:

Add 10 µl compound per well.

(2) Enzyme Mix:

Stock	Final Conc (in 50 µl assay volume)	Per 20 µl mix (1 reaction)	Per 600 reactions
DEPC H ₂ O		17.06 µl	10236 µl
1 M HEPES, pH 7.5	20 mM	0.5 µl	300 µl
1 M MgCl ₂	5 mM	0.25 µl	150 µl
100 mM DTT	1 mM	0.5 µl	300 µl
100 µM UTP	0.5 µM	0.25 µl	150 µl
100 µM ATP	1 µM	0.5 µl	300 µl
10 µM CTP	0.08 µM	0.4 µl	240 µl
10 µM GTP	0.025 µM	0.125 µl	75 µl
BSA, 10 mg/ml	0.05 mg/ml	0.25 µl	150 µl
HCV RdRp NS5B d ₂₁ BK (500 µg/ml or ~7.5 µM)	24 nM	0.16 µl	96 µl

Total: 20 µl

12 ml

- 10 Add 20 µl enzyme mix into each well of the assay plate. Incubate compound and enzyme at room temperature for 15 minutes.

(3) Template mix – prepare ahead

[0060] Spin down a tube of RNA (5 µg/tube stored in 75% ethanol and 0.3 M sodium acetate) in a microcentrifuge for 20 minutes. at 4°C. One tube is enough

- 15 for 1 – 1.5 plates. Remove as much ethanol from the tube as possible by inverting the tube. Be gentle, pellet RNA may not adhere to the tube. Vacuum dry the RNA. Resuspend the RNA by adding 1 ml of DEPC water, close the cap of the tube tightly. To dissolve RNA, incubate RNA solution on ice for ~60 minutes and gently vortex. Spin briefly to ensure all RNA solution is down to the bottom of the
- 20 tube before opening cap. Gently transfer RNA solution into a 5 ml or larger tube. Add another 3 ml of DEPC water (total 4 ml of volume).

Add the following volumes of reagents

Stock	Final concentration	Per 20 μ l mix (1 reaction)	Per 600 reactions
RNAse-free water		2.98 μ l	1788 μ l
Hepes, 1M	20 mM	0.5 μ l	300 μ l
RNAse Inhibitor (40 μ /ml)	0.4 U/ μ l	0.5 μ l	300 μ l
33 P-GTP 3000 Ci/mmol, 10 μ Ci/ μ l (3.3 μ M)	0.025 μ M	0.0125 μ l	7.5 μ l
POF	3 nM	16 μ l	9600 μ l

Add 20 μ l template mix per reaction (*i.e.* 20 ng of pOF per reaction or ~3 nM)

(4) Incubate reaction at room temperature (22-25°C) for 2 hours.

5 (5) Stop reaction by adding 50 μ l of 170 mM EDTA.

Final concentration of EDTA is 85 mM.

(6) Prewet filters of Millipore multiscreen assay plate by adding 200 μ l of 0.5 M sodium phosphate buffer, pH 7.0 into each well. Let stand at room temperature for 2 – 3 minutes.

10 (7) Place the multiscreen filter plate onto a Millipore Manifold and turn on vacuum to allow buffer to flow through. Turn off vacuum. Transfer 80 μ l of the reaction product into each well of the filter plate. Let stand for 2 – 3 minutes. Turn on vacuum to filter reaction product.

(8) Turn off vacuum. Add 200 μ l of 0.5 M sodium phosphate buffer, pH 7.0 into
15 each well to wash filter. Turn on vacuum.

Repeat step (8) three more times.

(9) Remove polypropylene bottom. Spot dry filter at the bottom with paper towel. Air dry filter plate on a bench for 1 hour. Add 40 μ l Super Mix scintillant. Seal top of the plate with a tape. Place plate into a Packard carrier or micro-beta carrier.

20 (10) Count plate using a Packard Topcount or micro-beta counter. Program 10 for 33 P in Top count or 33 P program in micro-beta.

[0061] Percent inhibition is calculated after background subtraction as a percent reduction of activity relative to the positive control (average value of the plate excluding the negative controls). For the primary screen hits were chosen as
25 showing ≥ 75 % inhibition.

[0062] See, Ferrari et al. 1999, J. Virology 73:1649-1654: "Characterization of soluble Hepatitis C virus RNA-dependent RNA polymerase expressed in E. coli" and Takamizawa et al 1991, J. Virology 65:1105-1113: "Structure and characterization of the Hepatitis C virus genome isolated from human carriers, both
5 are hereby incorporated by reference."

[0063] The compounds of the present invention inhibited Hepatitis C polymerase as summarized in Table 1:

Table 1

Example	HCV pol IC50 (μ M)
1	0.33
2	0.44
3	0.06
4	0.08

[0064] The ability of the compounds of the present invention to inhibit Hepatitis C
10 virus replicon constitutively expressed in a human liver cell line was established by the following experimental procedure:

[0065] Clone A cells (licensed from Apath, LLC) are derived from Huh-7 cells (human hepatoma cell line) and constitutively express of the HCV replication proteins with concomitant amplification the HCV replicon (1b) genome. Cells are
15 maintained and passaged in DMEM/10% FCS/1 mg/ml G418 (Geneticin from Gibco #11811-023; other media components as described below in "elisa media"). Care should be taken to maintain cell monolayers at a subconfluent state by 1:3 or 1:4 passages every 3-4 days. The replicon is extremely sensitive to the cellular metabolism/proliferation state and replicon copy number will rapidly decline in
20 confluent monolayers (resting cells). Under ideal conditions each cell has, on average, 1000 copies of the HCV replicon genome.

Reagents:

[0066] Elisa media:

Dulbecco's Modified Eagle Media (DMEM) (Gibco #12430-047)

25 2% Fetal Calf Serum (FCS) (HyClone #SH30070.03)

1X pen/strep (Gibco #15140-122)

1X non-essential amino acids (NEAA) (Gibco #11140-050)

no G418

Glutaraldehyde (Fisher #02957-4)

TWEEN-20, 10% (Roche #1332465)

TRITON X-100 (Sigma #T-8787)

Superblock in PBS (Pierce #37515)

NS5a monoclonal antibody (Virostat #1873)

5 Goat antimouse-HRP monoclonal antibody (BioRad #172-1011)

3,3',5,5' tetramethylbenzidine (TMB) substrate (Sigma #T-0440)

[0067] Compound Dilution/Cell Plating:

Drug Plate Preparation (Mother Plate)

10 μ l of compounds (in DMSO) are added to column 3 of the mother plate. 5 μ l of DMSO are added to the remaining columns. Mother plates are set aside until ready for serial dilution to be performed.

Control Drugs

[0068] Drug and Cell Addition:

[0069] The process for each plate involves:

15 Prepare cell plates (daughter plates) by adding 52 μ l of Elisa media to each well.

In Mother plates, serially transfer 50 μ l/well from column 3 through column 12.

Transfer 8 μ l from mother plate to daughter plates (all 96 wells).

Place daughter plates in incubator until cells are prepared.

20 Harvest Clone A cells and plate directly into daughter plates at 0.7×10^5 cells/ml, 100 μ l/well.

All plates are incubated at 37°C in 5% CO₂ for 3 days.

[0070] Elisa Assay:

Remove media from 96-well plates (cells should be ca 80% confluent) by flicking into sink.

25 Add 130 μ l/well 1X PBS + 0.05% glutaraldehyde.

Incubate 37°C for 1 hour.

Remove by flicking into sink.

Wash 3X with 300 μ l/well PBS, shaking for 5 minutes each wash. Remove by flicking into sink.

30 Add 130 μ l/well PBS + 0.05% TWEEN-20 + 0.1% TRITON X-100.

Incubate 37°C for 10 minutes.

Remove by flicking into sink.

Add 300 μ l/well Superblock in PBS.

Incubate 37°C for 1 hour.

Remove by flicking into sink.

5 Wash 3x with 300 μ l/well PBS, shaking 5 minutes each wash. Remove by flicking into sink.

During last wash, make a 1:100 dilution of NS5a Monoclonal-antibody (Mab) in Superblock + 0.02% TWEEN-20.

After last wash, add 50 μ l/well diluted Mab.

Incubate 37°C for 1 hour.

10 Remove by flicking into sink.

Wash 3X with 300 μ l/well PBS + 0.02% TWEEN-20, shaking 5 minutes each wash.

Remove by flicking into sink.

15 During last wash, make a 1:500 dilution of goat antimouse-HRP Mab in Superblock + 0.02% TWEEN-20.

After last wash, add 50 μ l/well diluted Mab.

Incubate 37°C for 1 hour.

Remove by flicking into sink.

20 Wash 5X with 300 μ l/well PBS + 0.02% TWEEN-20, shaking 5 minutes each wash. Remove by flicking into sink.

Wash 3X with 300 μ l/well PBS, shaking 5 minutes each wash. Remove by flicking into sink.

After last wash, add 130 μ l/well room temperature TMB substrate.

Incubate until blue color develops.

25 Add 130 μ l/well 1N HCl to stop reaction (color turns from blue to yellow).

Read plates with O.D. 450 filter.

ANALYSIS OF RESULTS: IC₅₀ (μ M); IC₅₀ (μ g/ml); % Inhibition

REFERENCE COMPOUNDS: Interferon- α 2; 4-30 U/ml IC₅₀

30 [0071] The following non-limiting specific examples are included to illustrate the synthetic procedures used for preparing compounds of the Formula (A). In these examples, all chemicals and intermediates are either commercially available or can be prepared by standard procedures found in the literature or are known to those skilled in the art of organic synthesis.

Example 1**[(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid****5-Bromo-2-methylaniline**

5

[0072] The mixture of iron powder (9.31g, 167 mmol) and NH_4Cl (2.48g, 46.3 mmol) in water (50 mL) was refluxed for 30 minutes. To this hot mixture was added 4-bromo-2-nitrotoluene (10 g, 46.3 mmol) slowly and then the reaction mixture was refluxed for 48 hours. The mixture was cooled to room temperature and extracted with EtOAc (3 x 100 mL). The organic solution was washed with H_2O (3 x 200 mL) and brine (200 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (silica, 15% EtOAc in hexanes) to give 7.9g (92%) of title compound as a pale yellow oil. ^1H nuclear magnetic resonance (NMR) (CDCl_3): 300 MHz δ 6.88 (m, 1H), 6.81 (m, 2H), 3.63 (bs, 2H), 2.09 (s, 3H).

15

5-Bromo-2-methylphenylhydrazine Hydrochloride

[0073] To a suspension of 5-bromo-2-methylaniline (4.80g, 25.8 mmol) in concentrated HCl (16 mL) was added dropwise a solution of sodium nitrite (1.96 g, 28.4 mmol) in water (10 mL) over 30 minutes at 0°C . To the mixture was added dropwise a solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (17.46g, 77.4 mmol) in concentrated HCl (15 mL) over 50 minutes. After stirring for 1 hour at 0°C , the reaction mixture was basified with 50% NaOH (30 mL). The mixture was further diluted with water (20 mL) and treated with another 50% NaOH (10 mL) and then crushed ice (100 g). The reaction mixture was extracted with ether (3 x 100 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4 , and filtered. The filtrate was acidified by adding an anhydrous solution of HCl in ether (1 N in ether, 31 mL, 31 mmol). The precipitate was collected and dried under reduced pressure to give 4.57 g (75%) of title compound as a white amorphous solid. ^1H NMR (DMSO): 300 MHz δ 10.31 (bs, 3H), 8.11 (bs, 1H), 7.12 (s, 1H), 7.06 (m, 2H), 2.14 (s, 3H).

25

30

4-Bromo-7-methyl Tryptophol

[0074] To a solution of 5-bromo-2-methylphenylhydrazine hydrochloride (4.57 g, 19.2 mmol) in 30% aqueous tetrahydrofuran (THF) (100 mL) at 0°C was added

35

dropwise a solution of 2,3-dihydrofuran (1.60 mL, 21.2 mmol) in THF (10 mL). After stirring for 2 h at 0°C and 12 hours at room temperature, the reaction mixture was diluted with ether (100mL). The organic solution was washed with saturated NaHCO₃ (2 x 100 mL) and brine (100 mL), dried (Na₂SO₄) and concentrated. The residue was dissolved in ethylene glycol (30 mL), treated with ZnCl₂ (5.76 g, 42.2 mmol), and heated at 170°C for 4 hours. The reaction mixture was cooled down to room temperature and 6 N HCl (100 mL) was added. The mixture was extracted with ether (3 x 100 mL) and washed with water (200 mL) and brine (200 mL). The organic solution was dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (silica, 40% EtOAc in hexanes) to give 1.22 g (25%) of title compound as a light brown oil. ¹H NMR (CDCl₃): 300 MHz δ 8.23 (bs, 1H), 7.18 (d, *J* = 7.65 Hz, 1H), 7.08 (d, *J* = 2.16 Hz, 1H), 6.81 (d, *J* = 7.65 Hz, 1H), 3.95 (t, *J* = 6.42 Hz, 2H), 3.27 (t, *J* = 6.42 Hz, 2H), 2.40 (s, 3H), 1.69 (bs, 1H).

5-Bromo-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid Ethyl Ester

[0075] To a solution of 4-bromo-7-methyl tryptophol (1.12 g, 4.41 mmol) and ethyl butyrylacetate (0.71 mL, 4.41 mmol) in CH₂Cl₂ (20 mL) was added BF₃•OEt₂ (0.56 mL, 4.41 mmol) dropwise at room temperature. The solution was stirred for 2 hours and then washed with saturated aqueous NaHCO₃ (15 mL) and brine (15 mL). The organic phase was dried (Na₂SO₄) and filtered through a pad of silica gel. The filter cake was washed with additional CH₂Cl₂ and the combined organic layer was evaporated to provide 1.62 g (93%) of title compound as a white solid. ¹H NMR (CDCl₃): 300 MHz δ 9.33 (bs, 1H), 7.11 (d, *J* = 7.65 Hz, 1H), 6.76 (d, *J* = 7.65 Hz, 1H), 4.19 (m, 2H), 4.03 (m, 1H), 3.90 (m, 1H), 3.15 (m, 2H), 3.03 (d, *J* = 16.6 Hz, 1H), 2.89 (d, *J* = 16.6 Hz, 1H), 2.43 (s, 3H), 2.08 (m, 1H), 1.96 (m, 1H), 1.38 (m, 1H), 1.27 (t, *J* = 7.14 Hz, 3H), 1.18 (m, 1H), 0.87 (t, *J* = 7.29 Hz, 3H).

5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid Ethyl Ester

[0076] 5-Bromo-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid ethyl ester (1.27 g, 3.22 mmol) and CuCN (0.433 g, 4.83 mmol) was dissolved in N-methyl-2-pyrrolidinone (15 mL) and the solution was divided into the 4 microwave reaction vessels (3.75 mL each). The reaction vessels were heated in

microwave at 220 °C for 15 minutes. The reaction mixtures in 4 vessels were combined and then diluted with water (30 mL). The crude mixture was extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with brine (100 mL), dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (silica, 20% EtOAc in hexanes) to give 0.959 g (88%) of title compound as a white solid. ¹H NMR (CDCl₃): 300 MHz δ 9.75 (bs, 1H), 7.33 (d, *J* = 7.52 Hz, 1H), 6.93 (d, *J* = 7.52 Hz, 1H), 4.21 (m, 2H), 4.11 (m, 1H), 4.03 (m, 1H), 3.08 (t, *J* = 5.52, 2H), 2.99 (d, *J* = 4.17 Hz, 2H), 2.57 (s, 3H), 2.06 (m, 2H), 1.42 (m, 1H), 1.26 (t, *J* = 7.16 Hz, 3H), 1.18 (m, 1H), 0.88 (t, *J* = 7.32 Hz, 3H).

10 **5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid**

[0077] To a solution of 5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid ethyl ester (0.959 g, 2.82 mmol) in THF/MeOH (7 mL/15 mL) was added 1 N NaOH (5.64 mL, 5.64 mmol). The reaction mixture was stirred at ambient temperature overnight. Most of THF/MeOH was removed under reduced pressure and the resulting mixture was acidified with 1 N HCl. The mixture was extracted with EtOAc (3 x 30 mL). The combined organic phase was washed with brine (60 mL), dried over Na₂SO₄ and concentrated to provide 0.868 g (99%) of title compound as a white solid. ¹H NMR (acetone-d₆): 300 MHz δ 10.37 (bs, 1H), 7.35 (d, *J* = 7.50 Hz, 1H), 7.03 (d, *J* = 7.50 Hz, 1H), 4.05 (m, 2H), 3.08-2.91 (m, 4H), 2.54 (s, 3H), 2.09 (m, 2H), 1.45 (m, 1H), 1.03 (m, 1H), 0.84 (t, *J* = 7.26 Hz, 3H).

15

20

25 **[(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid**

[0078] Preparative HPLC using CHIRALPACK-AD (250 x 20 mm) and 10% isopropyl alcohol in heptane (0.1% trifluoroacetic acid (TFA)) as eluant gave (R) and (S) enantiomers of 5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid as white solids. HRMS (ESI) [M+H]⁺ calculated for C₁₈H₂₁N₂O₃ 313.1547, found 313.1545 (R enantiomer) and 313.1547 (S enantiomer); Chiral HPLC HP 1100 with spiderlink CHIRALPACK-AD, 250 x 4.6 mm, isopropyl alcohol/heptane containing 0.1% TFA (10:90), 1.0 mL/minutes, DAD 215 nm; t_R = 6.98 minutes (R enantiomer), 9.37 minutes (S enantiomer).

30

- [0079] Alternatively, [(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid can be obtained by resolution with cinchonine according to the following procedure. (±)-5-Cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid (6.4 g, 20.5 mmol) and cinchonine (5.9 g, 20.0 mmol) were dissolved in a mixture of 2-butanone (125 mL) and water (5 mL) with heating. The clear solution was stirred and allowed to cool to room temperature overnight. The resulting solid was isolated, washed with 10 mL of 2-butanone, and dried to give 2.4 g (20% yield, >98% e.e.). The mother liquor was concentrated and dissolved again in a mixture of 2-butanone (100 mL) and water (1.5 mL) with heating. The solution was stirred and allowed to cool to room temperature overnight. The resulting solid was isolated, washed with 10 mL of 2-butanone, and dried to give a second crop of salt: 2.3 g (18% yield, >98% e.e.). The two crops (total 4.7 g) were combined and treated with 50 mL of 1N HCl and 100 mL of ethyl acetate. The ethyl acetate layer was washed with 1N HCl (30 mL) and water (50 mL). The aqueous layers were combined and extracted with ethyl acetate (50 mL). This ethyl acetate layer was washed with water (50 mL). The combined ethyl acetate layers were dried over sodium sulfate, filtered, and concentrated in vacuo to give 2.25 g. This material was triturated with 10 mL of ethyl acetate and the precipitate was collected, rinsed with 5 mL of ethyl acetate, and dried to give 1.27 g (e.e. >98%). The mother liquor was concentrated to a volume of 5 mL and the newly formed precipitate was collected, rinsed with 2 mL of ethylacetate and dried. A second crop of 0.4 g was obtained with an e.e. of >99%. The mother liquor was concentrated and gave a third crop of 0.5 g with an e.e. of >99%.
- [0080] The absolute configuration of the compound of Example 1 was determined by single crystal X-ray crystallography of the 4-bromobenzyl amide derivative, which was prepared as described below.

1-(R)-N-(4-Bromo-benzyl)-2-(5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)-acetamide

- [0081] To a solution of 1-(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid (20.0 mg, 0.064 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 15.0 mg, 0.077

mmol) and 1-hydroxybenzotriazole (10.4 mg, 0.077 mmol) in DMF (4 mL) was added N,N-diisopropylethylamine (67 μ l, 0.384 mmol) followed by 4-bromobenzylamine hydrochloride (17.1 mg, 0.077 mmol) at room temperature. The reaction mixture was stirred for 20 hours at ambient temperature. Water (5 mL) was added to the mixture and the resulting mixture was extracted with EtOAc (3 x 10 mL). The combined organic phase was washed with brine (20 mL), dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (silica, 40% EtOAc in hexanes) to give 27 mg (88%) of title compound as a white solid. The solid was crystallized from EtOAc for X-ray crystallography. Mp = 173-175 °C; ¹H NMR (CDCl₃): 300 MHz δ 10.15 (bs, 1H), 7.33 (m, 3H), 6.97 (m, 2H), 6.88 (m, 1H), 4.42 (dd, *J* = 11.2, 4.6 Hz, 1H), 4.29 (dd, *J* = 11.2, 4.6 Hz, 1H), 4.03 (m, 2H), 3.11-2.95 (m, 4H), 2.24 (s, 3H), 2.07 (m, 1H), 1.91 (m, 1H), 1.35 (m, 2H), 0.89 (t, *J* = 5.4 Hz, 3H); HRMS (ESI) [M+H]⁺ calculated for C₂₅H₂₇BrN₃O₂ 480.1281, found 480.1285.

15 Example 2

[(R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid
5-Bromo-2-fluoroaniline

- 20 [0082] Iron powder (9.3g, 0.166mM) and ammonium chloride (1.7g, 0.032mM) were stirred in water (42ml) at 100°C for 30 minutes. Commercially available 2-nitro 4-bromo fluorobenzene (9.2g, 0.42mM) was added drop wise to the above solution over a period of 45 minutes. The reaction was stirred at 100°C for an additional five hours. Water was removed in vacuo. The resultant crude solution
- 25 was stirred in ethyl acetate (100mL) for 20 minutes and the organic solution was decanted off. This wash was repeated two more times. The organic layers were combined, dried (MgSO₄), passed through a plug of SiO₂, and concentrated to afford 4.2g (53% yield) of the desired product as a red oil. The product was used without further purification. NMR (CHCl₃) δ 3.78 (bs, 2H); 6.65-7.07(m, 3H).
- 30 [0083] See, Courtin, A. Helv. Chim. Acta. 66, 1, (1983), hereby incorporated b reference.

5-Bromo-2-fluorophenylhydrazine

- [0084] A solution of sodium nitrate (0.49g, 0.007mM) in water (1.5ml) was added drop wise to a vigorously stirred heterogeneous solution of 5-bromo-2fluoroaniline (1.4g) in concentrated HCl(aq)(3.5ml) over a 30 minutes period at 0°C. Tin (II) chloride dihydrate (4.5g, 0.02mM) in concentrated HCl(aq) (3.5ml) was added drop wise to the above solution over a period of 30 minutes. After the addition, the solution was allowed to stir at 0°C for one hour. The reaction solution was basified (pH>7) by slowly adding a solution of 50% aqueous NaOH to the reaction mixture. The water layer was washed with diethyl ether (3x). The organic layers were combined, dried (MgSO₄), and concentrated. The resultant solid was thoroughly washed with hexanes. The undissolved solid was captured on filter and further washed with hexanes to afford 0.81g (54% yield) of the desired product as an off-white solid. NMR (CHCl₃) δ 5.45 (bs, 1H); 6.80-6.86(m, 2H); 7.25-7.28 (m, 1H).
- [0085] See, McKittrick, B. et al., J. Heterocyclic Chem. 27, 2151 (1990), hereby incorporated by reference.

4-Bromo-7-fluoro Tryptophol

- [0086] 2,3 Dihydrofuran (2.0ml, 0.026mM) was added to a solution of 5-bromo-2-fluorophenyl hydrazine (4.43g, 0.21mM) in dry THF (40ml) at 0°C. Concentrated HCl(aq) (2.0ml) was added to the mixture and the reaction was allowed to warm to room temperature and stirred overnight. THF was removed in vacuo. The crude residue was taken up in water and washed with ethyl acetate (3x). The organic layers were combined, dried (MgSO₄), and concentrated to afford 4.2g of a mixture of the mono and di-adducts as a red oil. This crude mixture was used without further purification in the next step.
- [0087] Zinc chloride (5.4g, 0.39mM) and the crude mixture were stirred in ethylene glycol at 160°C for three hours. The reaction was cooled and diluted with 10% HCl (aq) (50ml). The aqueous layer was washed with ethyl acetate (3x). The organic layers were combined, dried (MgSO₄), and concentrated. The product was purified by using silica gel flash chromatography (mobile phase: 3:2/hexanes: ethyl acetate) to afford 1.2g (yield: 21%) of the desired product as an off-white solid. NMR(CHCl₃) δ 3.26 (t, 2H, 6.3Hz); 3.96(t, 2H, 6.4Hz); 6.75 (m, 1H); 7.15(m, 2H); 8.54(bs, 1H).

**5-Bromo-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic
Acid Ethyl Ester**

[0088] BF₃-etherate (0.74ml, 0.0059mM) was added to a solution of 4-bromo-7-
5 fluorotryptophol (1.0g, 0.0039mM) and ethyl butyrylacetate (0.93ml, 0.0059mM)
in dry dichloromethane (15ml). This reaction was stirred for three hours at room
temperature. Saturated NaHCO₃ (aq) (15ml) was added to quench the reaction.
The solution was washed with DCM (2X). The organic layers were combined,
dried (MgSO₄), passed through a plug of SiO₂, and concentrated to afford 1.02g
10 (66% yield) of the desired product as an off-white solid. NMR (CHCl₃) δ 0.87 (t,
3H, 7.38Hz); 1.44(m, 1H); 1.28(t, 3H, 7.14Hz); 1.39(m, 1H); 1.93(m, 1H); 2.03(m,
1H); 2.91m(m, 1H); 3.06(m 1H); 3.15(m, 2H), 3.91(m, 1H); 4.03(m, 1H), 4.22(m,
2H); 6.72(m, 1H); 7.09(m, 1H); 9.50(s, 1H).

**5-Cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic
15 Acid Ethyl Ester**

[0089] The above ester (1.02g, 0.026mM) was dissolved in N-Methyl
pyrrolidinone (12ml). This solution was distributed equally into four Personal
Chemistry microwave reaction vessels. CuCN (0.085g, 0.0096mM) was added
20 into each reaction vessel. The reaction vessels were heated, under microwave
conditions, at 220°C for 15 minutes. The reaction solutions were combined and
diluted with water (30ml). The aqueous layer was washed with ethyl acetate (3X).
The organic layers were combined, dried (MgSO₄), and concentrated. The product
was purified by SiO₂ flash chromatography to afford 0.81g (92% yield) of the
25 desired product as an off-white solid. NMR (d₆-DMSO) δ 0.78 (t, 3H); 0.86(m,
2H); 1.0(t, 3H); 1.29(m, 2H); 1.92(m, 2H); 2.76(d, 1H); 2.86(t, 2H); 3.02(d, 1H);
3.9(m, 4H); 7.07(m, 1H); 7.5(m, 1H); 11.94(s, 1H).

**5-Cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic
30 Acid**

[0090] 1N NaOH(aq) (4.6ml) was added to a solution of the above ester (0.8g,
0.0023mM) in 1:1/ MeOH: THF (10ml) and stirred at room temperature overnight.
THF and MeOH were removed in vacuo. The residue was diluted with brine
(10ml), acidified with (pH<2) concentrated HCl(aq), and washed with ethyl acetate
35 (3X). The organic layers were combined, dried (MgSO₄), and concentrated to

afford 0.61 g (82% yield) of the desired product as a white solid. NMR (d_6 -DMSO) δ 0.95 (t, 3H, 5.4Hz); 1.23(m, 1H); 1.42(m, 1H); 2.05(m, 1H); 2.99-3.13 (m, 4H); 3.99(m, 1H), 4.11(m, 2H); 6.90(m, 1H); 7.39(m, 1H); 9.45(s, 1H).

5 **[(R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid**

[0091] Preparative HPLC using CHIRALPACK-AD (250 x 20 mm) and 10% isopropyl alcohol in heptane (0.1% TFA) as eluant gave (R) and (S) enantiomers of 5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid as
10 white solids. Chiral HPLC HP 1100 with spiderlink CHIRALPACK-AD, 250 x 4.6 mm, isopropyl alcohol/heptane containing 0.1% TFA (10:90), 1.0 mL/minutes, DAD 215 nm; t_R = 6.1 minutes (R enantiomer), 8.3 minutes (S enantiomer).

[0092] The absolute configuration of the compound of Example 2 was determined by single crystal X-ray crystallography of the 4-bromobenzyl amide derivative.

15 **1-(R)-N-(4-Bromo-benzyl)-2-(5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)-acetamide**

[0093] The procedure described for Example 3 was followed starting from 1-(R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid. 1H
20 NMR (d_6 -DMSO) δ 0.79 (t, 3H, 5.4Hz); 0.94(m, 1H); 1.31(m, 1H); 1.96(m, 2H); 2.75 (d, 1H, 10.2Hz); 2.91(m, 3H), 4.03(m, 2H); 4.21(d, 2H, 4.5Hz); 7.09(m, 3H); 7.37(d, 2H, 6.0Hz); 7.52(m, 1H); 8.22(t, 1H, 6.0Hz); 11.93(s, 1H); MS: M-H: 482.1; CHN for $C_{24}H_{23}BrFN_3O_2$ - Theory: C: 59.51, H: 4.79, N: 8.68 Found: C: 59.53, H: 4.86, N: 8.66.

25 **Example 3**

[(R)-5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid
4,7-Dichloro-Tryptophol

30 [0094] To a solution of 2,5 dichlorophenylhydrazine hydrochloride (20.4g 0.11 mol) in THF (80 mL) at 0°C was added dropwise a solution of 2,3-dihydrofuran (10.5 mL, 0.14 mol), water (15 mL) and HCL concentrated (5 mL). After stirring for 4 hours, the reaction mixture was diluted with ether (100 mL). The organic solution was washed with saturated NaCl (2 x 50 mL) and dried (Na_2SO_4) and
35 concentrated. The residue was dissolved in ethylene glycol (60 mL), treated with

ZnCl₂ (34.6 g, 0.25 mol), and heated at 140°C for 8 hours. The reaction mixture was cooled down to room temperature and 10% HCl was added. The mixture was extracted with ethyl acetate (3 x 75 mL) and washed with brine. The organic solution was dried over Na₂SO₄ and concentrated. The residue was purified by
5 flash chromatography (silica gel 60, EtOAc:Hexane 3:1) to give 10.4 g (39%) of title compound as a light brown oil. ¹H NMR (CDCl₃): 300 MHz δ 8.35 (bs, 1H), 7.16 (d, *J* = 2.1 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 1H), 3.95 (t, *J* = 6.3 Hz, 2H), 3.25 (t, *J* = 6.3 Hz, 2H), 1.49 (bs, 1H).

10 **5,8 dichloro -1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid Ethyl Ester**

[0095] To a solution of 5,8 dichloro tryptophol (4.25 g, 18.55 mmol) and ethyl butyrylacetate (4.37 mL, 27.63 mmol) in CH₂Cl₂ (40 mL) was added BF₃•OEt₂ (3.50 mL, 27.63 mmol) dropwise at room temperature. The solution was stirred for
15 2 hours and then washed with saturated aqueous NaHCO₃ (30 mL) and brine and concentrated. The oil was then purified by flash chromatography (silica gel 60, EtOAc:Hexane 4:1) to yield 1.5 g (32%). ¹H NMR (CDCl₃): 300 MHz δ 9.55 (bs, 1H), 7.03 (d, *J* = 8.10 Hz, 1H), 6.95 (d, *J* = 8.10 Hz, 1H), 4.3 (m, 2H), 4.02 (m, 1H), 3.89 (m, 1H), 3.01 (m, 2H), 2.99 (m, 1H), 2.92 (m, 1H), 2.01 (m, 2H), 1.28
20 (m, 5H), 0.88 (t, *J* = 7.30 Hz, 3H).

5,8 dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid

[0096] To a solution of 5,8 dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid ethyl ester (1.2 g, 3.24 mmol) in EtOH (35 mL) was added 1
25 N NaOH (7 mL). The reaction mixture was stirred at 50°C for 6 hours. Most of EtOH/NaOH was removed under reduced pressure and the resulting mixture was purified on HPLC to yield a white solid 0.730g (66%). ¹H NMR (CDCl₃): 300
MHz δ 9.12 (bs, 1H), 7.03 (d, *J* = 8.26 Hz, 1H), 6.96 (d, *J* = 8.26 Hz, 1H), 4.04 (m, 2H), 3.14 (m, 2H), 3.06 (m, 2H), 2.03 (m, 2H), 1.42 (m, 1H), 1.21 (m, 1H), 0.89 (t, *J*
30 = 7.34 Hz, 3H).

[(R)-5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid

- [0097] Preparative HPLC using CHIRALCEL OJ (250 x 20 mm) and 3% isopropyl alcohol in heptane (0.1% TFA) as eluant gave (S) and (R) enantiomer of 5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid as a white solid. Chiral HPLC – HP 1100 with spiderlink; CHIRALCEL OJ, 250 x 4.6 mm, isopropyl alcohol/heptane (containing 0.1% TFA) = 3:97, 1.0 mL/minutes, DAD 215 nm; t_R = 10.2 minutes (S enantiomer), 15.7 minutes (R enantiomer).

Example 4

[(R)-5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid
4-Bromo-3-fluoro-6-nitrotoluene

- [0098] To a stirred solution of 4-bromo-3-fluorotoluene (10 g, 52.9 mmol) in H_2SO_4 (100 mL) was added KNO_3 (5.34 g, 52.9 mmol) at 0°C. After stirring overnight at room temperature, the reaction mixture was poured into ice (200 g) and extracted with EtOAc (3 x 300 mL). The organic solution was washed with brine (200 mL), dried (Na_2SO_4), and concentrated to give 12.35 g (100%) of title compound as a pale yellow oil. 1H NMR ($CDCl_3$): 300 MHz δ 8.29 (d, J = 6.30 Hz, 1H), 7.12 (d, J = 8.61 Hz, 1H), 2.60 (s, 3H).

5-Bromo-4-fluoro-2-methylaniline

- [0099] The mixture of Iron powder (17.8 g, 318 mmol) and NH_4Cl (5.10 g, 95.4 mmol) in water (100 mL) was refluxed for 30 minutes. To this hot mixture was added 4-bromo-3-fluoro-6-nitrotoluene (18.6 g, 79.5 mmol) slowly and then the reaction mixture was refluxed for 48 hours. The mixture was cooled to room temperature and extracted with EtOAc (3 x 200 mL). The organic solution was washed with H_2O (3 x 300 mL) and brine (300 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (silica, 20% EtOAc in hexanes) to give 11.7 g (72%) of title compound as a pale yellow solid. 1H NMR ($CDCl_3$): 300 MHz δ 6.82 (m, 2H), 3.49 (bs, 2H), 2.11 (s, 3H).

5-Bromo-4-fluoro-2-methylphenylhydrazine Hydrochloride

[0100] To a suspension of 5-bromo-4-fluoro-2-methylaniline (11.2 g, 54.9 mmol) in concentrated HCl (35 mL) was added dropwise a solution of sodium nitrite (4.17 g, 60.4 mmol) in water (20 mL) over 30 minutes at 0°C. To the mixture was added dropwise a solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (37.2 g, 165 mmol) in concentrated HCl (45 mL) over 1 hour. After stirring for 2 hours at 0 °C, the reaction mixture was basified with 50% NaOH (50 mL). The mixture was further diluted with water (50 mL) and treated with another 50% NaOH (20 mL) and then crushed ice (200 g). The reaction mixture was extracted with ether (3 x 200 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4 , and filtered. The filtrate was acidified by adding an anhydrous solution of HCl in ether (2 N in ether, 42 mL, 82.5 mmol). The precipitate was collected and dried under reduced pressure to give 9.92 g (71%) of title compound as a pale yellow solid. ^1H NMR (DMSO): 300 MHz δ 10.18 (bs, 3H), 7.98 (bs, 1H), 7.21 (m, 2H), 2.16 (s, 3H).

4-Bromo-5-fluoro-7-methyl Tryptophol

[0101] To a solution of 5-bromo-4-fluoro-2-methylphenylhydrazine hydrochloride (4.75 g, 18.6 mmol) in 20% aqueous THF (100 mL) at 0°C was added dropwise a solution of 2,3-dihydrofuran (1.55 mL, 20.4 mmol) in THF (10 mL). After stirring for 2 hours at 0 °C and 12 hours at room temperature, the reaction mixture was diluted with ether (100mL).

[0102] The organic solution was washed with saturated NaHCO_3 (2 x 100 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated. The residue was dissolved in ethylene glycol (50 mL), treated with ZnCl_2 (5.58 g, 40.9 mmol), and heated at 170 °C for 4 hours. The reaction mixture was cooled down to room temperature and 6 N HCl (100 mL) was added. The mixture was extracted with ether (3 x 100 mL) and washed with water (200 mL) and brine (200 mL). The organic solution was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (silica, 40% EtOAc in hexanes) to give 1.52 g (30%) of title compound containing inseparable impurities (< 20%) as a light brown oil. ^1H NMR (CDCl_3): 300 MHz δ 8.68 (bs, 1H), 7.06 (d, J = 2.4 Hz, 1H), 6.76 (d, J = 9.63 Hz, 1H), 3.92 (t, J = 6.48 Hz, 2H), 3.21 (t, J = 6.48 Hz, 2H), 2.35 (s, 3H), 2.27 (bs, 1H).

5-Bromo-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid Ethyl Ester

[0103] To a solution of 4-bromo-7-methyl tryptophol (400 mg g, 1.47 mmol) and ethyl butyrylacetate (0.28 mL, 1.76 mmol) in CH₂Cl₂ (5 mL) was added BF₃•OEt₂ (0.22 mL, 1.76 mmol) dropwise at room temperature. The solution was stirred for 2 hours and then washed with saturated aqueous NaHCO₃ (5 mL) and brine (5 mL). The organic phase was dried (Na₂SO₄) and concentrated. The residue was purified by flash chromatography (silica, 15% EtOAc in hexanes) to give 496 mg (82%) of title compound as a pale yellow solid. Mp = 137-138 °C; ¹H NMR (CDCl₃): 300 MHz δ 9.73 (bs, 1H), 6.76 (d, *J* = 10.1 Hz, 1H), 4.21 (m, 2H), 4.05 (m, 1H), 3.91 (m, 1H), 3.05-2.89 (m, 4H), 2.53 (s, 3H), 2.07 (m, 1H), 1.92 (m, 1H), 1.38 (m, 1H), 1.30 (t, *J* = 6.98 Hz, 3H), 1.21 (m, 1H), 0.89 (t, *J* = 7.08 Hz, 3H).

5-Cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid Ethyl Ester

[0104] 5-Bromo-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid ethyl ester (496 mg, 1.20 mmol) and CuCN (162 mg, 1.81 mmol) was dissolved in N-methyl-2-pyrrolidinone (6 mL) and the solution was divided into the 2 microwave reaction vessels (3.0 mL each). The reaction vessels were heated in microwave at 220°C for 15 minutes. The reaction mixtures in 2 vessels were combined and then diluted with water (10 mL). The crude mixture was extracted with EtOAc (3 x 20 mL). The combined organic phase was washed with brine (50 mL), dried over Na₂SO₄ and concentrated. The residue was purified by flash chromatography (silica, 25% EtOAc in hexanes) to give 404 mg (94%) of title compound as a white solid. ¹H NMR (DMSO): 300 MHz δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, *J* = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, *J* = 10.3 Hz, 1H), 2.83 (t, *J* = 3.9 Hz, 1H), 2.72 (d, *J* = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, *J* = 5.41 Hz, 3H).

5-Cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic Acid

[0105] To a solution of 5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid ethyl ester (404 mg, 1.13 mmol) in THF/MeOH (2.5 mL/5 mL) was added 1 N NaOH (2.26 mL, 2.26 mmol). The

reaction mixture was stirred at ambient temperature overnight. Most of THF/MeOH was removed under reduced pressure and the resulting mixture was acidified with 1 N HCl. The mixture was extracted with EtOAc (3 x 10 mL). The combined organic phase was washed with brine (20 mL), dried over Na₂SO₄ and concentrated to provide 341 mg (91%) of title compound as a white solid. ¹H NMR (DMSO): 300 MHz δ 12.02 (bs, 1H), 11.33 (bs, 1H), 7.00 (d, *J* = 9.00 Hz, 1H), 3.96 (m, 2H), 2.95 (d, *J* = 10.3 Hz, 1H), 2.83 (t, *J* = 3.9 Hz, 1H), 2.72 (d, *J* = 10.3 Hz, 1H), 2.54 (s, 3H), 1.99 (m, 2H), 1.28 (m, 1H), 0.85 (m, 1H), 0.79 (t, *J* = 5.41 Hz, 3H).

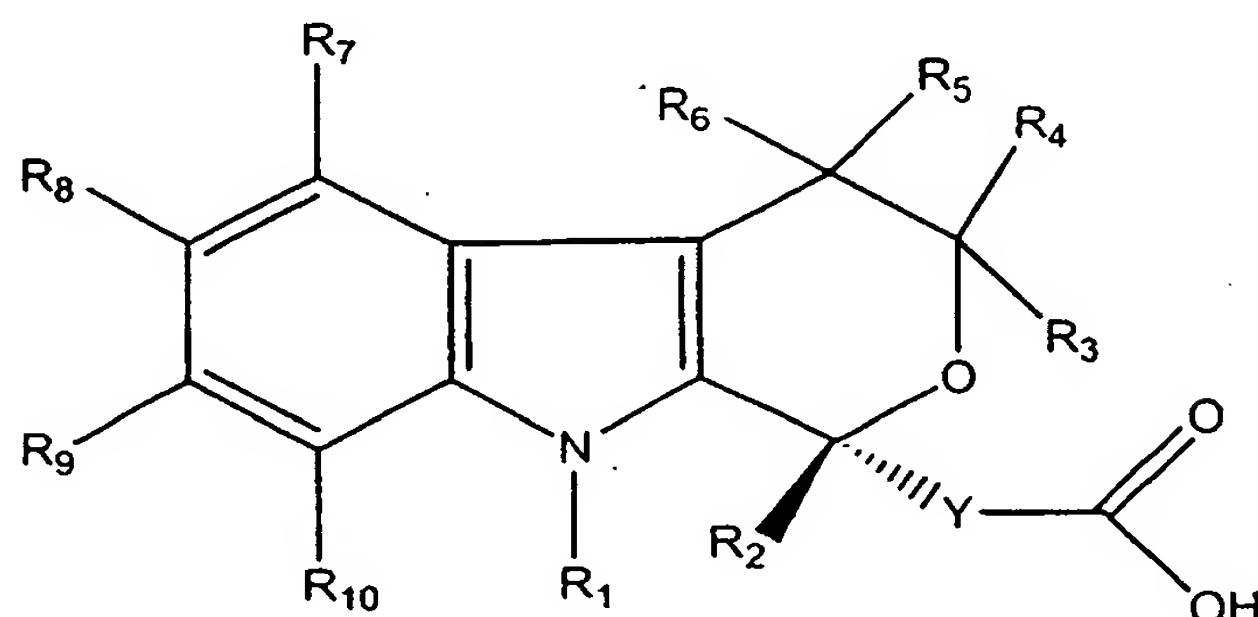
10 **[(R)-5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid**

[0106] Preparative HPLC using CHIRALPACK-AD (250 x 20 mm) and 10% isopropyl alcohol in heptane (0.1% TFA) as eluant gave (R) and (S) enantiomers of 5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indole-1-acetic acid as white solids. HRMS (ESI) [M+H]⁺ calculated for C₁₈H₂₀FN₂O₃ 331.1453, found 331.1447 (R enantiomer) and 331.1452 (S enantiomer); Chiral HPLC HP 1100 with spiderlink CHIRALPACK-AD, 250 x 4.6 mm, isopropyl alcohol/heptane containing 0.1% TFA (10:90), 1.0 mL/minutes, DAD 215 nm; t_R = 7.19 minutes (R enantiomer), 9.27 minutes (S enantiomer).

[0107] Alternatively, [(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid can be obtained by resolution with cinchonine according to the procedure described for example 1.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a compound of a formula:



wherein:

R₁ is H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or an arylalkyl or an alkylaryl of 7 to 12 carbon atoms;

R₂ is H, a straight chain alkyl of 1 to 12 carbon atoms, a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, an alkoxyalkyl of 2 to 12 carbon atoms, an arylalkyl or alkylaryl of 7 to 12 carbon atoms, a cyanoalkyl of 1 to 8 carbon atoms, an alkylthioalkyl of 2 to 16 carbon atoms, a cycloalkyl-alkyl of 4 to 24 carbon atoms, a substituted or unsubstituted aryl, or a heteroaryl;

R₃ – R₄ are independently H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, a substituted or unsubstituted aryl, furanylmethyl, arylalkyl or alkylaryl of 7 to 12 carbon atoms, alkynyl of 2 to 7 carbon atoms, or R₅ and R₆ together with the ring carbon atom to which they are attached form a carbonyl group;

R₇ – R₁₀ are independently H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbons atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, a substituted or unsubstituted aryl, a substituted or unsubstituted heteroaryl, furanylmethyl, arylalkyl or alkylaryl of 7 to 12 carbon atoms, alkynyl of 2 to 7 carbon atoms, phenylalkynyl, alkoxy of 1 to 8 carbon atoms, arylalkoxy of 7 to 12 carbon atoms, alkylthio of 1 to 8 carbon atoms,

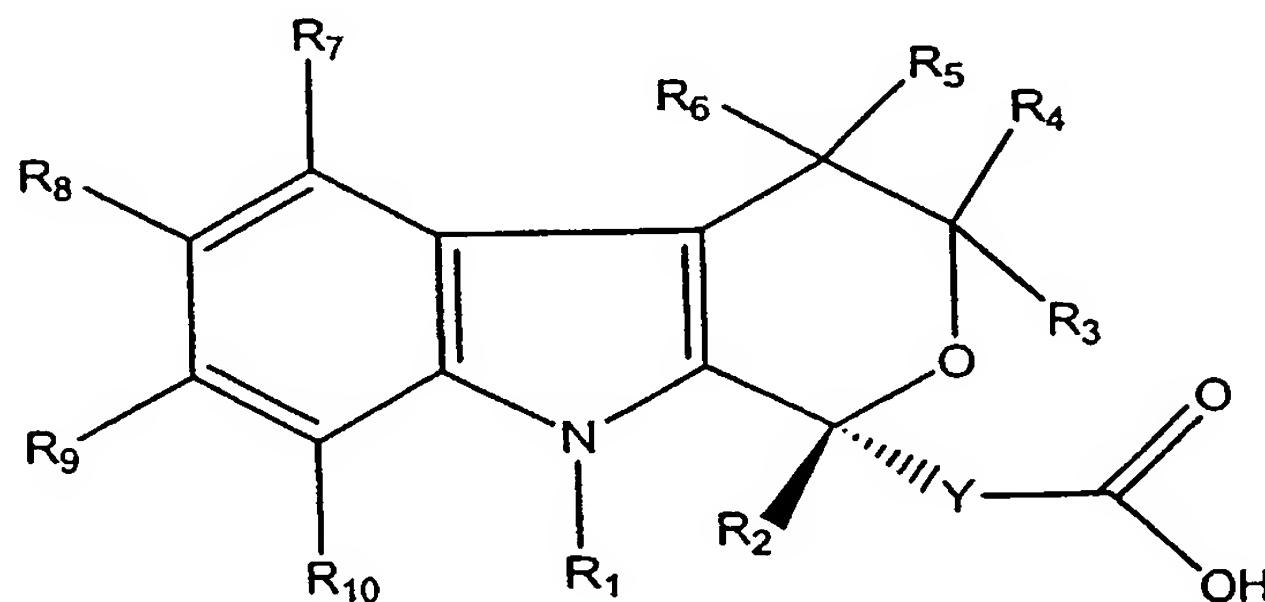
trifluoromethoxy, trifluoroethoxy, trifluoromethylthio, trifluoroethylthio, acyl of 1 to 6 carbon atoms, COOH, COO-alkyl, $\text{CONR}_{11}\text{R}_{12}$, F, Cl, Br, I, CN, CF_3 , NO_2 , alkylsulfinyl of 1 to 8 carbon atoms, alkylsulfonyl of 1 to 6 carbon atoms, pyrrolidinyl, or thiazolidinyl;

$\text{R}_{11} - \text{R}_{12}$ are independently H, straight chain alkyl of 1 to 8 carbon atoms, branched alkyl of 3 to 12 carbon atoms, cycloalkyl of 3 to 12 carbon atoms, a substituted or unsubstituted aryl or heteroaryl;

Y is a bond, CH_2 , CH_2CH_2 , aryl, or R_2 and Y together with the ring carbon atom to which they are attached may additionally form a spirocyclic cycloalkyl ring of 3 to 8 carbon atoms; or

a crystalline form or a pharmaceutically acceptable salt thereof; and
a pharmaceutically acceptable carrier.

2. The pharmaceutical composition of claim 1 comprising a compound of the formula:



wherein:

R_1 is H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or an arylalkyl of 7 to 12 carbon atoms;

R_2 is H, a straight chain alkyl of 1 to 12 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, an alkoxyalkyl of 2 to 12 carbon atoms, an arylalkyl of 7 to 12 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, or heteroaryl;

$R_3 - R_6$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbons atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, furanylmethyl, an arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or R_5 and R_6 together with the ring carbon atom to which they are attached form a carbonyl group;

$R_7 - R_{10}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbons atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups, furanylmethyl, an arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms, phenylalkynyl, an alkoxy of 1 to 6 carbon atoms, an arylalkoxy of 7 to 12 carbon atoms, an alkylthio of 1 to 6 carbon atoms, trifluoromethoxy, trifluoroethoxy, trifluoromethylthio, trifluoroethylthio, an acyl of 1 to 6 carbon atoms, a carboxy group, $\text{CONR}_{11}\text{R}_{12}$, F, Cl, Br, I, CN, CF_3 , NO_2 , an alkylsulfinyl of 1 to 6 carbon atoms, an alkylsulfonyl of 1 to 6 carbon atoms;

$R_{11} - R_{12}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an aryl substituted with one to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups;

Y is CH_2 , CH_2CH_2 , or aryl; or

a crystalline form or a pharmaceutically acceptable salt thereof; and
a pharmaceutically acceptable carrier.

3. The pharmaceutical composition of Claim 1 wherein the compound of the formula is 100% Isomer A.
4. The pharmaceutical composition of Claim 1 wherein the compound of the composition has a ratio of Isomer A to Isomer B of at least about 9:1.
5. The pharmaceutical composition of Claim 1 a ratio of Isomer A to Isomer B ratio of at least about 8:1.
6. The pharmaceutical composition of Claim 1 wherein the compound of the composition a ratio of Isomer A to Isomer B of at least about 7:1.
7. The pharmaceutical composition of Claim 1 wherein the Isomer A in the composition is selected from the group consisting of:

[(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid;

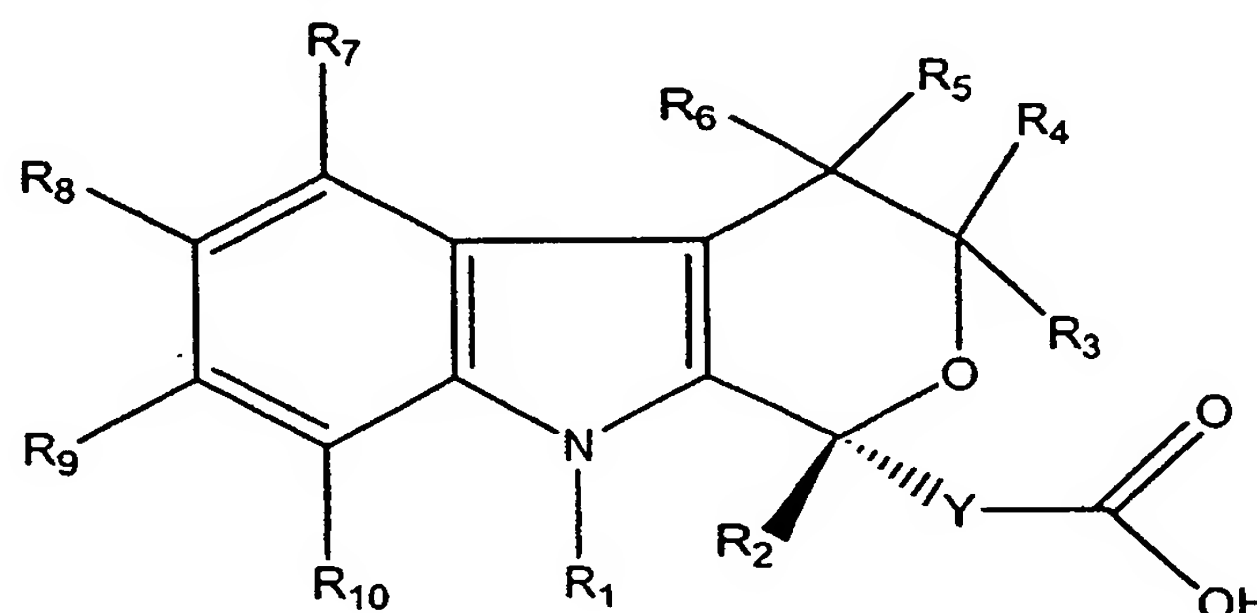
[(R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid;

[(R)-5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid; and

[(R)-5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid.

8. The pharmaceutical composition of Claim 1 wherein R₂ is n-propyl, (s)-sec-butyl, or cyclobutyl.

9. The pharmaceutical composition of claim 1 wherein:



R₁ is H;

R₂ is H, or a straight chain alkyl of 1 to 4 carbon atoms;

R₃ – R₆ are H;

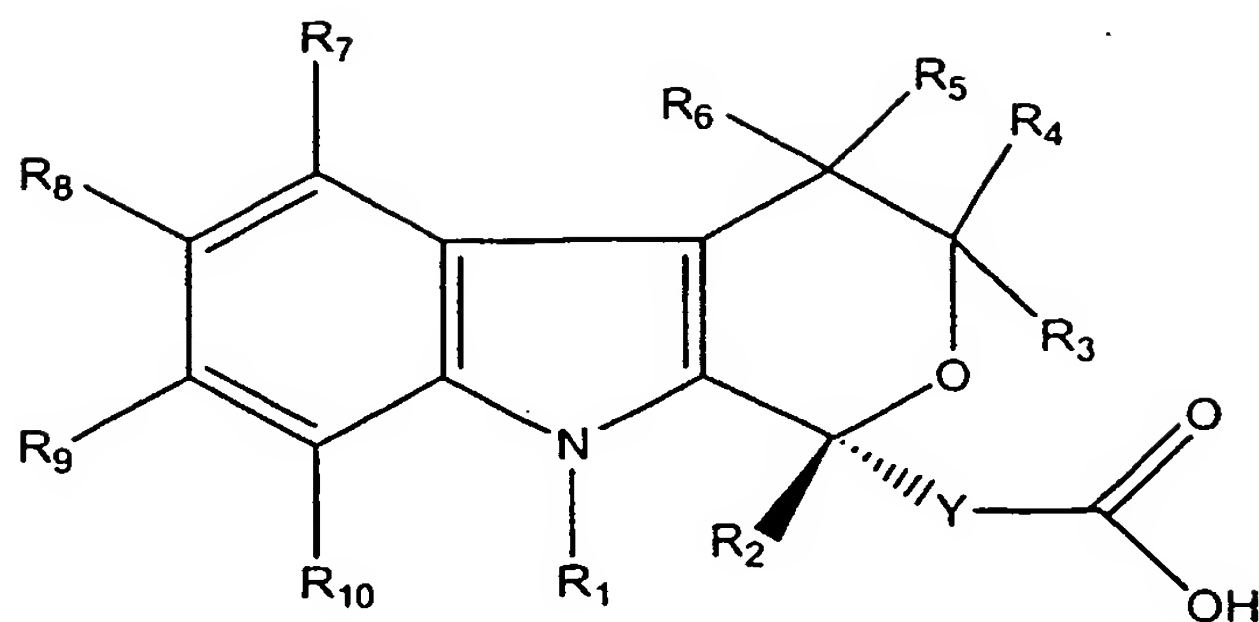
R₇ – R₁₀ are independently H, a straight chain alkyl of 1 to 3 carbon, F, Cl, or CN;

Y is CH₂; or

a crystalline form or a pharmaceutically acceptable salt thereof; and

a pharmaceutically acceptable carrier.

10. A compound of a formula:



wherein:

R_1 is H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or an arylalkyl of 7 to 12 carbon atoms;

R_2 is H, a straight chain alkyl of 1 to 12 carbon atoms, a branched alkyl of 3 to 12 carbon atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, an alkoxyalkyl of 2 to 12 carbon atoms, an arylalkyl of 7 to 12 carbon atoms, a cycloalkyl-alkyl of 4 to 24 carbon atoms, a substituted or unsubstituted aryl, or heteroaryl;

$R_3 - R_6$ are independently H, a straight chain alkyl of 1 to 8 carbon atoms, a branched alkyl of 3 to 12 carbons atoms, a cycloalkyl of 3 to 12 carbon atoms, an alkenyl of 2 to 7 carbon atoms, a substituted or unsubstituted aryl, furanylmethyl, arylalkyl or arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms;

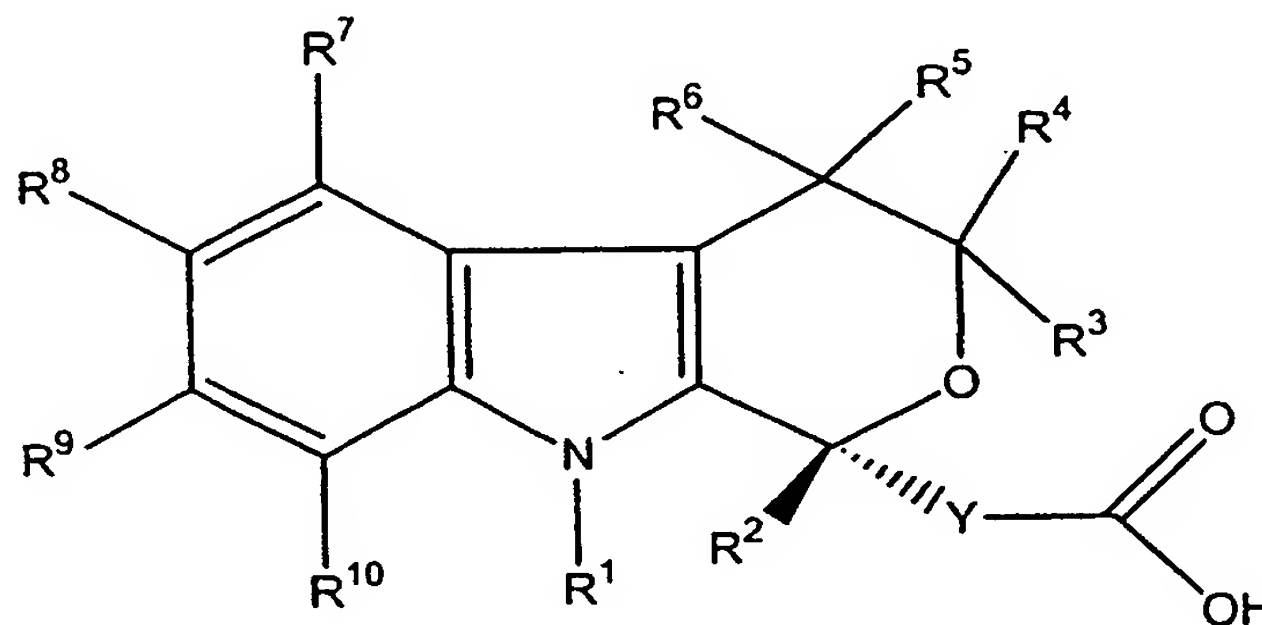
$R_7 - R_{10}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbons atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups, furanylmethyl, an arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms, phenylalkynyl, an alkoxy of 1 to 6 carbon atoms, an arylalkoxy of 7 to 12 carbon atoms, an alkylthio of 1 to 6 carbon atoms, trifluoromethoxy, trifluoroethoxy, trifluoromethylthio, trifluoroethylthio, an acyl of 1 to 6 carbon atoms, a carboxy group, $\text{CONR}_{11}\text{R}_{12}$, F, Cl, Br, I, CN, CF_3 , NO_2 , an alkylsulfinyl of 1 to 6 carbon atoms, an alkylsulfonyl of 1 to 6 carbon atoms;

$R_{11} - R_{12}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an aryl substituted with one to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups;

Y is CH_2 , CH_2CH_2 , or aryl; or

a crystalline form or a pharmaceutically acceptable salt thereof.

11. The compound of claim 10 wherein;



R_1 is H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or an arylalkyl of 7 to 12 carbon atoms;

R_2 is H, a straight chain alkyl of 1 to 12 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an alkynyl of 2 to 7 carbon atoms, an alkoxyalkyl of 2 to 12 carbon atoms, an arylalkyl of 7 to 12 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, or heteroaryl;

$R_3 - R_6$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbons atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an unsubstituted aryl or an aryl substituted with one to four groups, furanylmethyl, an arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms, or R_5 and R_6 together with the ring carbon atom to which they are attached form a carbonyl group;

$R_7 - R_{10}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbons atoms, a cycloalkyl of 3 to 10 carbon atoms, an alkenyl of 2 to 7 carbon atoms, an unsubstituted aryl or an aryl substituted with one

to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups, furanylmethyl, an arylalkyl of 7 to 12 carbon atoms, an alkynyl of 2 to 7 carbon atoms, phenylalkynyl, an alkoxy of 1 to 6 carbon atoms, an arylalkoxy of 7 to 12 carbon atoms, an alkylthio of 1 to 6 carbon atoms, trifluoromethoxy, trifluoroethoxy, trifluoromethylthio, trifluoroethylthio, an acyl of 1 to 6 carbon atoms, a carboxy group, $\text{CONR}_{11}\text{R}_{12}$, F, Cl, Br, I, CN, CF_3 , NO_2 , an alkylsulfinyl of 1 to 6 carbon atoms, an alkylsulfonyl of 1 to 6 carbon atoms;

$\text{R}_{11} - \text{R}_{12}$ are independently H, a straight chain alkyl of 1 to 6 carbon atoms, a branched alkyl of 3 to 10 carbon atoms, a cycloalkyl of 3 to 10 carbon atoms, an aryl substituted with one to four groups, an unsubstituted heteroaryl or a heteroaryl substituted with one to three groups;

Y is CH_2 , CH_2CH_2 , or aryl; or;

a crystalline form or a pharmaceutically acceptable salt thereof.

12. The compound of Claim 10 is selected from the group consisting:

[(R)-5-cyano-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid;

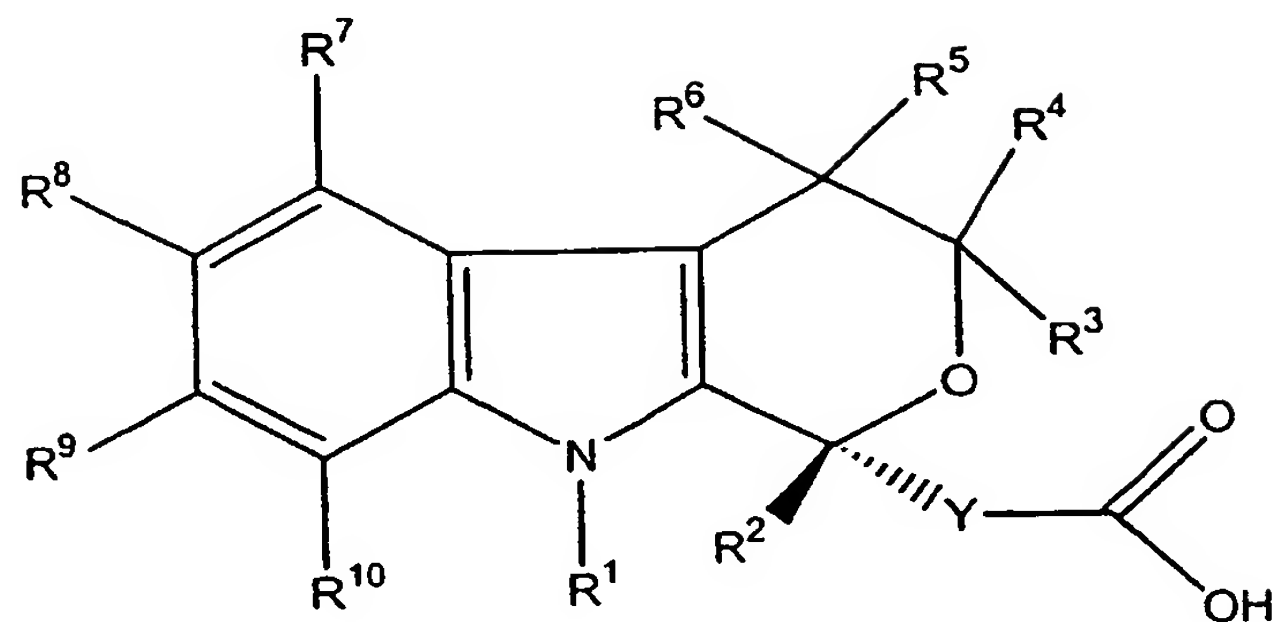
[(R)-5-cyano-8-fluoro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid;

[(R)-5,8-dichloro-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid; and

[(R)-5-cyano-6-fluoro-8-methyl-1-propyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl]acetic acid.

13. The compound of Claim 10 wherein R_2 is n-propyl, (s)-sec-butyl, or cyclobutyl.

14. The compound of claim 10 wherein:



R_1 is H;

R_2 is H, straight chain alkyl of 1 to 4 carbon atoms;

$R_3 - R_6$ are H;

$R_7 - R_{10}$ are independently H, straight chain alkyl of 1 to 3 carbon, F, Cl, or CN;

Y is CH_2 ; or

crystalline form; or a pharmaceutically acceptable salt thereof.

15. A method of obtaining the compound of claim 10 comprising:

- a. dissolving a racemic mixture of a compound with a chiral amine with heating to obtain a solution;
- b. stirring and cooling the solution of step (a) to obtain a first solid and a liquid;
- c. isolating, the solid of step (b) from the liquid of step (b);
- d. washing and drying the solid from step (c);
- e. repeating step (b) on the liquid of step (c) to obtain a second solid;
- f. combining the first and second solids and treating the combined solids with HCl and ethyl acetate to obtain an ethyl acetate layer and a liquid layer;
- g. washing the ethyl acetate layer of step (f) to obtain a liquid layer;
- h. combining the liquid layers from step (f) and step (g);
- i. extracting the combined layers from step (h) with ethyl acetate;
- j. washing the ethyl acetate layer from step (i) with water;
- k. drying, filtering and concentrating the ethyl acetate layer to obtain a solid;

l. triturating the solid of step (k) with ethyl acetate to obtain a precipitate and a liquid;

m. drying the precipitate obtained in step (g) to obtain a compound of claim 10; and

n. repeating steps (k) and (l) on the liquid of step (l) to obtain additional compound of claim 10.

16. The method of claim 15 wherein the chiral amine comprises an ephedrine hemihydrate or cinchomine.

INTERNATIONAL SEARCH REPORT

PCT/US 03/15823

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D491/04 A61K31/407 A61P31/12 //C07M7:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 17680 A (SEPRACOR, INC.) 16 September 1993 (1993-09-16) the whole document ---	1-6, 8-11, 14-16
X	US 4 604 469 A (CHRISTOFER A. DEMERSON) 5 August 1986 (1986-08-05) the whole document ---	10,11, 14-16
A	US 5 578 734 A (GIUSEPPE VECCHI) 26 November 1996 (1996-11-26) the whole document --- -/--	10,11, 14-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

19 August 2003

Date of mailing of the International search report

01/09/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Beslier, L

INTERNATIONAL SEARCH REPORT

PCT/US 03/15823

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUMBER L. G. ET AL.: "Etodolac (1,8-diethyl-1,3,4,9-tetrahydropyrano(3,4-b)indole-1-acetic acid): a potent antiinflammatory drug. Conformation and absolute configuration of its active enantiomer" JOURNAL OF MEDICINAL CHEMISTRY., vol. 29, no. 5, 1986, pages 871-874, XP002251535 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US ISSN: 0022-2623 the whole document ---	1-16
A	WO 02 00167 A (MEDINOX, INC.) 3 January 2002 (2002-01-03) claims 1,2,25,32,41,43 -----	1-16

INTERNATIONAL SEARCH REPORT

PCT/US 03/15823

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9317680	A	16-09-1993	AT	193648 T	15-06-2000
			AU	3806393 A	05-10-1993
			AU	707780 B2	22-07-1999
			AU	3999297 A	18-12-1997
			CA	2131992 A1	16-09-1993
			DE	69328838 D1	13-07-2000
			DE	69328838 T2	12-10-2000
			DK	727994 T3	07-08-2000
			EP	0727994 A1	28-08-1996
			JP	7506817 T	27-07-1995
			WO	9317680 A1	16-09-1993
			US	5561151 A	01-10-1996
<hr/>					
US 4604469	A	05-08-1986	US	4544757 A	01-10-1985
<hr/>					
US 5578734	A	26-11-1996	CH	685345 A5	15-06-1995
			IT	1269177 B	21-03-1997
<hr/>					
WO 0200167	A	03-01-2002	US	6355666 B1	12-03-2002
			US	6429223 B1	06-08-2002
			AU	7001001 A	08-01-2002
			CA	2414150 A1	03-01-2002
			EP	1296929 A2	02-04-2003
			WO	0200167 A2	03-01-2002
			US	2003088111 A1	08-05-2003